NITROGEN DEPOSITION AND THE SUSTAINABILITY OF LOWLAND HEATHLANDS IN BRITAIN

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FOR HENRY

Always understanding
Always there
Thank you

Abstract

Despite widespread conservation efforts, global heathland area has substantially decreased in recent decades. Heathland habitats require low nitrogen availability in order to persist. Over the past 150 years, however, nitrogen deposition has increased markedly. Early observational studies and research using artificial N applications have identified N deposition as the primary driver of heathland succession into grassland or woodland, and N enrichment is considered a threat to heathland sustainability. This study investigated soil fertility and vegetation composition at 25 lowland heathland sites in low rainfall regions of mainland Britain within a modelled wet N deposition range of 1.85 to 10.90 kg N ha^{-1} y^{-1}. A bioassay approach was used to quantify relationships between soil fertility and N deposition, heathland patch size and the management regimes. This study discovered significant positive relationships between N enrichment and *C. vulgaris* shoot mass, N and P concentrations. No relationship between N enrichment and N : P mass ratio was found suggesting no N induced shift to P limitation. It was determined that soil phosphomonoesterase activity was not up-regulated in response to N enrichment. This suggests that the soil P reserves are sufficient to satisfy demand under current N deposition loads. Heathland patch size was negatively related to *C .vulgaris* shoot dry-mass which was used as a proxy for soil fertility. Measured atmospheric ammonia concentrations were not related to *C. vulgaris* growth and shoot chemistry. No relationships were found between any variable tested and heathland vegetation composition suggesting that local factors, such as management intervention, may be substantial determinants of vegetation composition. This study presents relationships

between temperature at origin and *C. vulgaris* growth from populations located along a latitudinal gradient in Western Europe. The findings of this thesis have implications for current heathland management, and for future management under a climate change scenario.

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Abbreviations

N_W	-	Modelled inorganic wet N deposition (kg N ha^{-1} y^{-1})
N_D	-	Modelled inorganic dry N deposition (kg N ha^{-1} y^{-1})
N_T	-	Modelled total inorganic N deposition (kg N ha^{-1} y^{-1})
NH_3	-	Modelled ammonia-N emissions (µg m^3)
$[NH_3]$	-	Measured ammonia-N emissions (µg m^3)
W_W	-	Whole plant mass (mg)
S_W	-	Plant shoot mass (mg)
R_W	-	Plant root mass (mg)
R_W:S_W	-	Plant root : shoot ratio
$[N]_{shoot}$	-	N concentration in plant shoot (%)
$[P]_{shoot}$	-	P concentration in plant shoot (%)
$([N]:[P])_{shoot}$	-	Shoot nitrogen : phosphorus ratio
PME	-	Phosphomonoesterase
*p*NPP	-	*para*-nitrophenyl phosphate
TO_{min}	-	Minimum annual temperature at origin (°C)
TO_{mean}	-	Mean annual temperature at origin (°C)
TO_{max}	-	Maximum annual temperature at origin (°C)
T_0	-	Mean daily temperature (°C)
T_{+3}	-	Mean daily temperature + 3°C (°C)
T_{+6}	-	Mean daily temperature + 6°C (°C)

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General Introduction

1.1 Heathland ecology

Heathlands are areas of open country without tree growth or continuous grass turf, consisting primarily of dwarf-shrubs of the Ericaceae, as first defined by Graebner (1901) and Warming (1909). They developed in Europe around 4000 years ago as a result of forest clearance followed by grazing, burning and cutting the vegetation for fuel and fodder (Gimingham, 1972; Webb, 1986). Soils are typically podzols on sandy mineral layers, of low pH in the range 3.4 - 6.5, and are deficient in nutrients, particularly nitrogen, phosphorus and calcium (Gimingham, 1992; Webb, 1986). Heathland can occur at a range of altitudes, but lowland heathland communities are regarded as those below 300 m elevation. The vegetation is characterised by the dominance of one or more ericaceous shrubs, such as *Calluna vulgaris, Erica* spp., *Vaccinium* spp. or *Ulex* spp. (Rodwell, 1991).

There are some discrepancies between different definitions of heathland. The term 'heathland' has been used to describe lichen and bryophyte dominated communities, arctic and coastal habitats, and communities on acidic soils (McVean, 1964; McVean & Ratcliffe, 1962; Tansley, 1939; Watt, 1940). The indiscriminate use of the terms 'moor' and 'heath' has further confused any definitive designation (Gimingham, 1972). The criteria used to define heathland also vary between local and national datasets of heathland extent, thus an accurate catalogue of heathland cover within the UK is likely to be marred by discrepancies in its designation (Clifton & Keymer, 2002).

1.2 Heathlands and global change

Temperate heathlands are now under threat from global change. Sala *et al*. (2000) suggested that land-use change, climate change, increased N deposition, biotic exchange (deliberate or accidental introduction of species to a habitat) and the effect of increased atmospheric CO_2 on photosynthetic capacities are globally the most important determinants of biodiversity loss. Here biodiversity is defined as total species, genetic and habitat diversity. Land-use change is the driver that is expected to have the greatest effect on global biodiversity across the biomes studied, followed by climate change (Figure 1.1a). Elevated N deposition, however, is predicted to be the most influential driver of biodiversity loss in temperate forest zones by 2100 (Figure 1.1b).

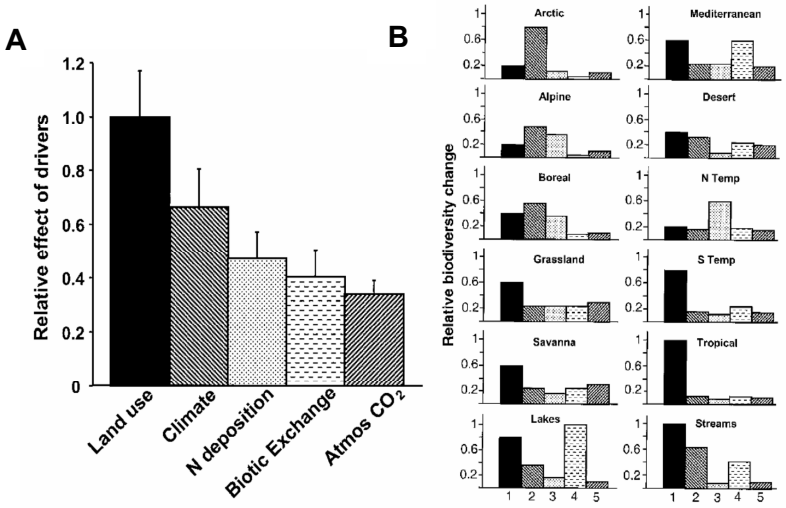


Figure 1.1 (A) The relative effect of five drivers of expected biodiversity change by 2100 across ten terrestrial and two freshwater biomes, calculated as the product of the expected change in drivers and the impact of each driver on biodiversity for each biome. Values are estimates of the averages for each biome. (B) The relative effect of each driver on biodiversity change for each biome. Bar 1 = land use, 2 = climate, 3 = N deposition, 4 = biotic exchange, 5 = atmospheric CO_2. N Temp and S Temp represent northern and southern temperate forest zones (Sala *et al.*, 2000).

Over the past 150 years the greatest rise in N deposition has been in northern hemisphere temperate ecosystems, with an average four-fold increase (Holland *et al*., 1999), although deposition began to fall post 1970 (Matejko *et al*., 2009). Nitrogen limited systems, such as heathlands, are strongly affected by N enrichment (Lee, 1998; Lee & Caporn, 1998). This has been reported to result in change of heathland into grassland (Heil & Diemont, 1983) or woodland (Köchy & Wilson, 2001), and is threatening heathland sustainability under a global change scenario.

1.3 Impact of nitrogen enrichment

1.3.1 Sources of nitrogen and phosphorus

Both natural and anthropogenic nitrogenous gases are emitted into the atmosphere through various means. Ammonia (NH_3) is volatilised from decomposing animal and plant matter. This is intensified in agricultural systems where the large quantities of animal excreta produced has led to substantial NH_3 emissions of 242 kt-N y^{-1} in the UK (NEGTAP, 2001; Misselbrook *et al*., 2000; Webb, 2001). An additional 46 kt-N y^{-1} is emitted from other non-agricultural sources, such as internal combustion engines (Sutton *et al*., 2000). NH_3 is converted to ammonium (NH_4^+) in solution. Nitrogen oxides (NO_x), which include nitric oxide (NO), nitrous oxide (N_2O) and nitrogen dioxide (NO_2) are released naturally from soils and sediments through denitrification and generated by atmospheric electrical processes such as lightning. Natural emissions contribute between 2 and 23% of the total budget (NEGTAP, 2001). Anthropogenic emissions totalling 488 kt-N y^{-1} in the UK derive from internal combustion processes in vehicle engines, and through industrial activities (Fowler *et al*, 2004; Goodwin *et al*., 2001;

NEGTAP, 2001). Nitric acid (HNO_3) is emitted primarily through industrial processes and is rarely emitted naturally.

Phosphorus occurs in the atmosphere as dust derived from soil and rock erosion. This is deposited in wind-blown dust and sand, and in volcanic emissions and natural fires (Newman, 1995; Sprent, 1987). However, there is a dearth of information on the quantities involved, and on the anthropogenic sources of P. Nevertheless, industrial processes, such as phosphate production, and discharges from coal and timber combustion, are considered to be significant contributors to atmospheric P loads (Graham & Duce, 1979).

1.3.2 Deposition of nitrogen in the British mainland

Nitrogenous pollutants are either wet deposited via precipitation events, or dry deposited via sediments or in gaseous form. There is little information on nitrogen deposition in Britain prior to 1950 as countrywide networks of pollution samplers were only developed subsequently (Eriksson, 1952; Fowler *et al*., 2004; Sutton *et al*., 1993). It is known, however, that over the past century average nitrogen deposition in temperate regions in the northern hemisphere increased substantially from 0.68 to 4.30 kg N ha^{-1} y^{-1} due to greater emissions from fossil fuel combustion, industrial and agricultural processes (Holland *et al.,* 1999*;* Pitcairn *et al.*, 1991; Pitcairn *et al*., 1995; Misselbrook *et al*., 2011). Between 1990 and 2005 total nitrogen deposition in Britain decreased from 369 Gg-N to 317 Gg-N (Matejko *et al*., 2009), due to active control measures, such as the 1999 Gothenburg Protocol and changes in industry and agricultural practices, and this reduction is expected to continue.

In mainland Britain the historical increases in deposition have not been geographically uniform producing marked spatial variation in nitrogen

deposition (Figure 1.2). Wet nitrogen deposition is strongly promoted at high elevation due to orographic cloud and high rainfall. For example, Crossley *et al*. (1992) found that although cloud water deposition represented only 25% of the hydrological input in an upland forest, pollutant concentrations in cloud water were between two and four times greater than in that of rainfall and hence comprised the major input source. This phenomenon is also evident in Snowdonia National Park, North Wales, which is subject to disproportionately high acidifying pollution despite receiving rainfall that is amongst the least polluted in Europe (Gritten, 1992). This is due to extremely high annual precipitation, sometimes exceeding 5000 mm y^{-1}, which deposits significant amounts of nitrogen onto the area.

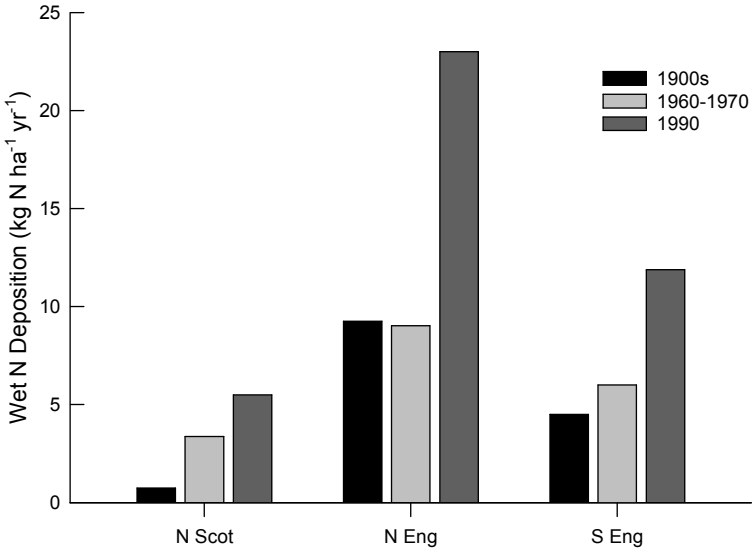


Figure 1.2 Changes in wet N deposition in northern Scotland, northern England and southern England between 1900 and 1990. Figure compiled from data obtained in a review by Pitcairn *et al*. (1995).

Spatial variability in N deposition also results from differences in the way that different N forms are emitted. For example, Skiba *et al*. (2004) demonstrated that from a point source of NH_3 emissions, such as poutry-manure fired

electric generators, NH_3 deposition declined substantially within 400 m of the source due to the low atmospheric residency time of NH_3 gas (Erisman *et al*., 1988). Wet deposited N is more diffusely distributed, and is usually deposited far away from the emission source, as demonstrated by Gritten (1992) in Snowdonia, North Wales, for example, who noted that peaks in pollutant deposition were correlated with strong easterly winds which transported pollutants emitted in industrial regions of the English Midlands.

1.3.3 Impact of N and P enrichment on heathlands

Increased atmospheric N deposition has driven species community change throughout temperate regions (Bobbink *et al*., 2010; Sala *et al*., 2000). This has been demonstrated, for example, in grasslands by Stevens *et al*. (2004), in woodlands by Bobbink *et al*. (2008) and Kirby *et al*. (2005), and in heathlands by Maskell *et al*. (2010) and Power *et al*. (1995). Nitrogen-limited ecosystems, such as heathlands, are particularly affected by N enrichment (Lee, 1998; Lee & Caporn, 1998). This is because many species that are adapted for growth in N limited habitats are slow-growing and stress-tolerant and are unable to compete with faster growing species, such as graminoids (Bobbink *et al*., 1998). Nitrogen enrichment in heathlands has resulted in local species extinctions by competitive exclusion, and ultimately heathland loss (McClean *et al*., 2011). As a result, increased nitrogen deposition has been identified as a substantial threat to heathland species richness and habitat sustainability (Aerts & Heil, 1993). Early evidence of this arose from field observations and, later, controlled manipulation experiments confirmed N enrichment as a causal agent of *C. vulgaris* loss.

1.3.3.1 Early studies

Tansley (1939) described heathlands as a "stage in the succession to forest", thus considering them a "subclimax" community. Gimingham (1972) suggested that halting this successional change requires some form of management, and that loss of heathland would usually follow the cessation of management. It was noted by Gimingham (1972) that this was more likely to happen in heathland where breaks in the *C. vulgaris* canopy are common. Watt (1955) observed the cyclical replacement of *C. vulgaris* with *Pteridium aquilinum* and the subsequent replacement of *P. aquilinum* with *C. vulgaris* on a dune heath in Britain and he attributed this to a four-phase growth development cycle seen in both *C. vulgaris* and *P. aquilinum*. This cycle is characterised by a pioneer, building, mature and degenerate growth phase, after which the individual plants become increasingly moribund and eventually perish (Figure 1.3).

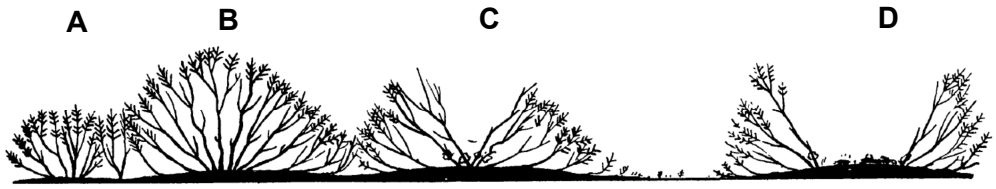


Figure 1.3 A diagram representing the four growth phases of *Calluna vulgaris* (a) pioneer, (b) building, (c) mature, and (d) degenerate (Watt, 1955).

In an observational 30 year study of a Dutch heathland Diemont & Heil (1984) predicted that, after the competitive exclusion of *C. vulgaris* by *Deschampsia flexuosa* following a *Lochmaea suturalis* (heather beetle) attack, it would take up to 25 years for a *C. vulgaris* dominated heath to return were the process cyclical. Diemont & Heil (1984) found that abiotic factors, such as minor differences in soil type, could determine whether cyclical or seral processes

occurred. They suggest, however, that discussion at this point is academic as management intervention is likely to interrupt the process before completion.

The link between *C. vulgaris* loss and increased soil fertility, rather than due to cyclical or successional changes, was first discovered in a series of papers originating in the Netherlands. Heil & Diemont (1983) noticed that, in response to repeated artificial applications of N at a rate of 28 kg N ha^{-1} y^{-1}, a heathland community underwent rapid succession into grassland, with *C. vulgaris* being nearly completely replaced by *Festuca ovina* after 12 years. Diemont & Heil (1984) suggested that the mineralisation of N after final growth-stage *C. vulgaris* die-off was the reason why graminoids became dominant. Aerts (1989) acknowledged that ericaceous species, such as *C. vulgaris* and *Erica* spp. show increased growth following nitrogen and phosphorus enrichment. Aerts & Berendse (1988), however, had demonstrated that the graminoid *Molinia caerulea* showed a much greater growth response to nitrogen enrichment than *Erica tetralix,* and so was able to exclude the slower growing ericoid, thus ultimately resulting in heathland loss.

1.3.3.2 Controlled experiments

A majority of subsequent research on the impact of N enrichment on heathlands focused on artificial, often ecologically unlikely, applications of N to observe the effects on one or more heathland patches. Power *et al*. (1995), for example, applied up to 15.4 kg N ha^{-1} y^{-1} in addition to the 18 kg N ha^{-1} y^{-1} background N deposition to a lowland heathland site in Surrey, UK, and observed significant stimulation in shoot growth, flowering and canopy density of *C. vulgaris* after five years. These relationships have also been demonstrated without artificial applications of N in studies of heathlands across an N deposition gradient. Pitcairn *et al*. (1995) found an approximately

linear increase of 0.045 mg shoot N g^{-1} dry-mass for each 1 kg N ha^{-1} y^{-1} deposited across 19 sites in mainland Britain. In a review of three studies using long-term artificial N applications from 0 - 120 kg N ha^{-1} y^{-1}, however, Power *et al*. (2004) noticed commonality between responses of foliar N concentrations in *C. vulgaris*, but found site-specific differences in biomass responses.

Therefore it is accepted that *C. vulgaris* shows a positive growth response to N enrichment (Aerts, 1989; Power *et al*., 1995; Uren *et al*., 1997). It is suggested, however, that graminoids invest more biomass in leaves than ericoids and so are able to eliminate ericaceous species by competitive exclusion (Aerts & Berendse, 1988; Hartley & Amos, 1999). This may be due to increased shading restricting shrub growth (Goldberg & Miller, 1990; Hautier *et al*., 2009; Tilman, 1988), although Dickson & Foster (2011) have argued that increased nutrient limitation is more likely. This might still not result in reduced *C. vulgaris* cover, however, because ericoids are able to outcompete faster - growing graminoids providing that they suffer no catastrophic damage due to frost, drought or *L. suturalis* attack (Aerts & Heil, 1993; Marrs, 1986 & 1993; Power *et al*, 1998). *Calluna vulgaris* experiences greater susceptibility to all these stresses with increased nitrogen deposition. Power *et al.* (1998), for example, noted that water loss from *C. vulgaris* shoots was increased under N enrichment, as was frost damage. Sæbø *et al*. (2001) found that N enrichment caused a change in the root:shoot ratio of *C. vulgaris,* resulting in reduced drought tolerance. Marrs (1986) suggested a direct link between reduced tolerance of *C. vulgaris* to drought or insect attack and the succession of heathland into woodland.

Nitrogen enrichment can also alter demand for other nutrients such as phosphorus. Rowe *et al.* (2008) found a significant positive relationship

between N deposition and P concentrations in *C. vulgaris* shoots at over 200 sites in Britain. This is supported by Jones & Power (2011), who demonstrated the same effect in *C. vulgaris* over a total N deposition range of 13.3 to 30.8 kg N ha^{-1} y^{-1}. This is contrary to research suggesting that N enrichment will promote a shift from N limitation to P limitation, which may arise because the demand for P is not satisfied by the relatively low P availability in acidic heathland soils or the low levels of atmospheric P deposition (Gress *et al*., 2007). Kirkham (2001) found a significant positive relationship between N deposition in the range 18.6 to 33.2 kg N ha^{-1} y^{-1} and shoot N : P ratios in six moorland species in Britain. He suggests that this is indicative of a shift from N to P limitation in response to N enrichment. Güsewell (2004), however, suggests that N : P ratios <10 indicate N limitation, and ratios >20 correspond to P limitation. The shoot N : P ratios found by Kirkham (2001) fall between these two critical ratios which, according to Güsewell (2004), means that it is uncertain whether the plants are N or P limited. A study by Pilkington *et al*. (2005c), on the other hand, found that long-term artificial additions of 40 to 120 kg N ha^{-1} y^{-1} increased shoot N : P ratios in moorland plants from 15.1 to 19.8, which is close to the P limitation threshold proposed by Güsewell (2004). It should be noted, however, that N deposition values of 120 kg N ha^{-1} y^{-1} are extremely unlikely to occur in Britain other than on managed farmland.

Greater soil phosphatase activities and mycorrhizal fungi colonisation in plant roots can increase nutrient availability, thus moderating N-induced P limitation (Pilkington *et al*., 2005b; Rowe *et al*., 2008). Phosphomonoesterase (PME) is an enzyme which catalyses the hydrolysis of phosphomonoesters in soil organic material (Jansson *et al*., 1988). PME activity is known to be promoted by increased nitrogen deposition (Hogan *et al*., 2010; Johnson *et al*., 1998 & 2010; Pilkington *et al*., 2005b), and is thought to result in an increase in

phosphorus availability, and subsequently reduced phosphorus limitation (Kritzler & Johnson, 2010). Fujita *et al*. (2010) found that artificial N additions enhanced PME activity associated with the root systems of eight grassland species in the Netherlands, and noted that species specific responses, with greater PME activity in grasses as opposed to forbs, may lead to species composition change beneficial to those adapted to P limitation. This proposal is reinforced by Kirkham (2001) and Roem *et al* (2002) who found that graminoids with vesicular-arbuscular fungal associations, such as *Molinia caerulea,* are better adapted to coping with phosphorus limitation and could outcompete ericoids in a phosphorus limited system.

Vesicular-arbuscular and ericaceous mycorrhizal fungi facilitate the absorption of nutrients in infertile soils and protect plants against stresses from toxic metals and organic acids, but they also produce phosphatase enzymes at the root surface, thus further promoting nutrient mobilisation and uptake (Bradley *et al*., 1981 & 1982; Leake & Miles, 1996; Read, 1983; Straker & Mitchell, 1986; Stribley *et al*., 1975). The level of mycorrhizal colonisation appears to be related to the form of nitrogen enrichment. There is no relationship between mycorrhizal colonisation and ammonium nitrate enrichment (Johansson, 2000), but there is a positive relationship between colonisation and ammonia enrichment (Heijne *et al*., 1994; Johansson, 2000). Diaz *et al*. (2006) suggest that above ground biomass is positively related to mycorrhizal colonisation in ericoids. Genney *et al*. (2001) found a reduction in mycorrhizal colonisation of *Nardus stricta* roots also correlated positively with the density of the plants, suggesting that less abundant species would be disadvantaged by lower mycorrhizal associations. Hofland-Zijlstra & Berendse (2009) found a reduction in mycorrhizal colonisation of ericoid roots in response to nitrogen

enrichment and suggested that this may be a result of increased shading by plants, especially graminoids, experiencing enhanced growth.

Nitrogen enrichment can also lead to soil acidification if leaching of base cations with NO_3^- exceeds leaching of NH_4^+ since H^+ ion concentration will increase (Skeffington & Wilson, 1988). This is particularly likely if the soil is saturated with N as leaching will be greatly increased (Pilkington *et al*., 2005a). Soil acidification can also occur if NH_4^+ is nitrified because two H^+ ions are yielded during the redox process. Lee *et al*. (1992) found no detrimental effect of soil acidification on *C. vulgaris* growth or mycorrhizal infection after three years. However, increases in soil acidity in heathlands and grasslands have been shown to result in reduced seed germination and growth of plants and an overall reduction in plant diversity (de Graaf *et al*., 2009; Maskell *et al*., 2010; Roem *et al*., 2002; Roem and Berendse, 2000). Owen & Marrs (2000) demonstrated that the intentional acidification of soil by the addition of elemental S successfully reduced the presence of graminoids while increasing the presence of ericoids and bare ground in a heathland in Suffolk, UK. However, if pH is reduced to 3.2, the germination of *C. vulgaris* seed is significantly lowered (Poel, 1949; Helsper & Klerken, 1984).

1.4 Effects of fragmentation and management

1.4.1 Effects of heathland fragmentation

Heathland once extended over several million hectares in Western Europe, but estimates suggest that only 350,000 ha now remain (Diemont *et al*., 1996). Within Nottinghamshire, heathland loss exceeded 85% between 1927 and 1970 (Glasson, 1987). Many heathland areas are subject to national and international protection in order to conserve the habitat. However, despite widespread conservation efforts, the average area of heathland sites has

continued to decrease, while the total number of sites has increased (Rose *et al*., 2000). One reason for this decrease in heathland area is the direct conversion of heathland to agricultural land, forest and urban/industrial areas (Rose *et al*., 2000). Another is vegetation succession resulting in the development of scrub and woodland. This has occurred in Dorset, for example, where between 1978 and 1987, the number of heathland patches increased from 142 to 151, but the total area decreased by 552 ha to 7925 ha, due to succession of heathland to woodland and scrub communities (Rose *et al*., 2000; Webb, 1990). A proportion of the remaining heathland in England and Wales has also been damaged by heavy grazing, neglect or otherwise inappropriate management (Bardgett *et al*., 1995). Restoration has been attempted, but once the heathland has undergone succession into grassland or woodland, it has proved difficult to successfully restore the heathland in the long term (Cox *et al*., 2008; Box *et al*., 2011; Pywell *et al*., 2011).

In the past, heathland loss has not been managed and has been associated with the disintegration of heathland areas into smaller fragments that have become increasingly isolated (Bender *et al*., 1998; Farig, 1997). The concept of the metacommunity, or a set of local communities that are linked by species dispersal, has yielded a number of theories that attempt to explain the effect of habitat isolation on communities (Leibold *et al*., 2004; Wilson, 1992). There are four main metacommunity paradigms. Firstly the patch-dynamic approach assumes that patches are identical and are subject to stochastic extinctions which can be counteracted by dispersal from other patches. The species-sorting paradigm takes into account abiotic factors which can affect the outcome of species interactions, therefore patches are assumed to be heterogeneous. The mass-effects theory suggests that species within patches are locally different in their competitive response, but regionally similar due to

their ability to compensate said competition through dispersal. Finally, the neutral paradigm assumes no difference between species, and suggests that differences in patches occur due to random extinctions. Recent reviews have, however, indicated that no single model can be applied to any individual region as the effects of isolation vary between species and locations (Driscoll & Lindenmayer, 2009).

Increased isolation due to fragmentation limits colonisation by species if their dispersal potential is too low, particularly if the species was removed initially by management techniques such as sod-cutting (Littlewood *et al*, 2009; Wallis De Vries & Ens, 2010). Piessens *et al*. (2005) suggest that increased patch isolation is a more important driver of heathland deterioration than reduced patch area, since local extinctions could be prevented by dispersal from nearby patches. Dieckhoff *et al* (2006) found that both the number of heathland plant species, and total number of species, increased with an increase in patch size, although the proportion of heathland species decreased. Different species vary in their response to habitat fragmentation due to differences in dispersal ability, and it is suggested that species with a persistent seed-bank, such as *C. vulgaris,* would be less affected by fragmentation than species with limited seed-longevity (Bossuyt & Hermy, 2003; Maurer *et al*., 2003; Thompson *et al*., 1997).

The increased ratio of patch edge to patch area in smaller heathlands may lead to ingress of nutrients from incoming litter, dust and ground water, ultimately leading to the encroachment of non-heathland species into the site, resulting in heathland loss. Piessens *et al*. (2006) reported an increase in soil fertility penetrating 8 m into heathland patches adjacent to cropland and woodland, producing substantial changes in vegetation composition at the habitat edge. *Calluna vulgaris* was less dominant at the edge in the sites

adjacent to woodland and had been replaced by graminoids, while *C. vulgaris* was absent at the edges in the sites adjacent to cropland. The same effect was observed among bryophytes on heathlands adjacent to woodland, and it was found that management by grazing or mowing could not mitigate this effect (Piessens *et al*., 2008). This trend is confirmed by a review of 25 published studies of fragmentation effects on various habitats by Bender *et al*. (1998), which concluded that species that occur in the interior, but which are absent in peripheral zones, will be detrimentally affected by habitat fragmentation. A reduction in grassland and heathland plant reproductive success was also reported to relate to increased fragmentation, possibly due to a reduction in the attractiveness of smaller plant populations to pollinating insects (Lennartsson, 2002; Sih & Baltus, 1987). Tsaliki & Diekmann (2010) for example found that total seed mass and germination rate per plant in two heathland *Genista* species was reduced with greater habitat fragmentation as defined by population size and isolation. Therefore it appears that increased fragmentation can facilitate N enrichment, leading to plant species invasions and resulting in a greater likelihood of local extinctions of heathland species, thereby accelerating heathland loss.

1.4.2 Effects of management

Heathland in Europe developed around 4000 years ago as a result of anthropogenic modifications to native woodlands, i.e. clearance and the subsequent use of the land for grazing stock or cutting vegetation for fuel and fodder, thus preventing the regeneration of the woodland (Gimingham, 1972; Webb, 1986). Consequently, in order for heathland to persist it requires some form of management (Britton *et al*., 2001). Traditional heathland management included grazing or sod-cutting for fuel (Heil & Aerts, 1993; Webb, 1986). The vegetation was also burnt to improve the forage for livestock (Webb, 1998).

Along with more recent practices, such as mowing, these traditional management tools are now used to conserve heathlands. Effective use of these techniques will result in reduced soil fertility, an enhanced establishment of desired species, such as *C. vulgaris*, and a reduction in the vigour of graminoids, trees and other heathland atypical species.

The impact of grazing by livestock is wholly dependent on the stocking density and on the animal used. For example, sheep are used because their small mouths allow them to avoid less palatable plants, such as ericaceous shrubs, while grazing by cattle is more generalised, effectively managing grasses but also consuming some ericoids in the process (Anderson & Radford, 1994; Grant *et al*., 1987). Too high a stocking density will reduce the presence of shrubs, lichens and bryophytes due to trampling, and increase the presence of bare soil and grazing-resistant graminoids (Bullock & Pakeman, 1997; Nolan *et al.*, 1995; Welch & Scott, 1995). At lower intensities grazing by sheep, or mixed grazing by sheep and deer, increases the presence of ericoids and reduces the presence of bare ground and species atypical to heathlands (Anderson & Radford, 1994; DeGabriel *et al*., 2011). Studies have shown that moderate grazing increases alpha and beta diversity of heathlands, and due to the spatial variability of animal grazing, can result in vegetation composition heterogeneity, which is critical to maintain diverse invertebrate assemblages (Usher & Thompson, 1993; Vandvik *et al*., 2005). Mowing on the other hand is not selective in the species affected, but can reduce soil fertility if the cuttings are removed (Barker *et al*., 2004; Britton *et al*., 2001; Diemont, 1994).

Burning is also an effective method used to reduce soil fertility as a result of direct soil nutrient modification (Forgeard & Frenot, 1996). Traditionally heathlands were burnt to provide a post-burn flush of vegetation for grazing

animals (Webb, 1998). Harris *et al.* (2011) suggest that many species show increased growth post-fire, but then their presence decreases subsequently. This is because burning removes soil N by downward percolation and surface runoff (Pilkington *et al*., 2007). *Calluna vulgaris* is the only species that has shown a sustained positive response to prescribed burning (Harris *et al*., 2011). The abandonment of applied burning over the last 50 years has resulted in the succession of some heathlands into *Betula* spp. or *Pinus sylvestris* L. dominated woodland (Miles, 1985; Miles & Young, 1980; Mitchell *et al*., 1999; Vandvik *et al*., 2005). If fire is uncontrolled or prescribed too frequently (<5 years), however, trees or grasses can establish more successfully than *C. vulgaris*, thus highlighting the inter-site variability in the success of burning (Ascoli & Bovio, 2010; Borghesio, 2009; Stevenson & Rhodes, 2000). Davies *et al*. (2010) suggests that burning areas with low soil moisture, as a result of prolonged drought for example, damaged *C. vulgaris* regeneration to a much greater extent than burning areas on moist peat, which is more effective at insulating against heat penetration.

The impacts of the above management techniques on soil fertility can be quantified by approximating the number of years of atmospheric N input that can be removed by one application. Continuous grazing at low densities for one year, and one application of prescribed burning can remove around five years of nitrogen deposition (Haerdtle *et al*., 2006). Sod-cutting or litter removal, on the other hand, can remove between the equivalent of 40 to 197 years of nitrogen deposition depending on N deposition levels (Haerdtle *et al*., 2006; Mitchell *et al*., 2000). If the management treatment is not applied frequently enough then there will be a net gain in nitrogen capital within the system. Initially, sod-cutting may appear to be the more favourable treatment to burning or grazing. However, as well as removing some of the seed-bank,

sod-cutting also removes ericaceous mycorrhizal fungi, which act to increase biomass production in ericoids, and also reduce mortality (Vergeer *et al*., 2006). The extent to which lowered soil fertility is maintained after sod-cutting is also variable, deterring managers from making the large financial investment necessary to apply this technique (Diemont, 1994; Heil & Aerts, 1993).

1.5 Potential effects of climate warming

Human-induced climate warming is likely to affect plant species diversity and climatic range distribution in the future (IPCC, 2007; Thuiller, 2004). There is now abundant evidence to suggest that species distributions have already responded to climate warming (Gregory *et al*., 2009; Hickling *et al*., 2006; Parmesan & Yohe, 2003). The annual mean temperature in northern Europe is expected to increase further by between 2.3°C and 5.3°C by 2099 (IPCC, 2007). The extent of heathland, which currently ranges latitudinally from Scandinavia to Morocco (Figure 1.4), may alter as a result of this temperature increase.

Figure 1.4 Map of the area in which lowland heathlands occur in Europe and Morocco. Areas are classified into five geographic groups which were expected to express broad biogeographic diversity based on criteria such as insularity, geographic barriers etc. Modified from Loidi *et al.* (2010).

Loidi *et al*. (2010) suggest that low temperature limits the occurrence of heathland in the northern most extent of its range, and summer drought limits its occurrence in the south. The impact of climate change on species ranges has often been assessed using bioclimatic envelope models (see Heikkinen *et al*., 2006). These models have had mixed success in projecting the direction of range changes under climate change, particularly in estimating the magnitude of such changes (Araújo *et al*., 2005). A model by Araújo *et al*. (2011) of the climatic suitability of 1883 European animal and plant species located in conservation areas predicted that 58 ± 2.3 % of species would lose areas of climatic suitability by 2080, effectively leading to reductions in their ranges. It is unclear, however, whether heathland species would be among those detrimentally affected.

Changes in temperature affect many ecosystem processes, including litter decomposition rates, nitrification, denitrification, nutrient uptake and photosynthetic performance and growth in plants, among others (Llorens *et al*., 2004; Rustad *et al*., 2001; Schmidt *et al*., 2004; Wessel *et al*., 2004; White *et al*., 1996). Peñuelas *et al* (2004) demonstrated that a 1°C two-year increase in temperature resulted in a 15% increase in above-ground plant biomass in an ericoid-dominated shrubland in the UK. Increased plant growth can be detrimental to heathlands as species with higher relative growth rates than *C. vulgaris*, such as *Pteridium aquilinum* (L. Kuhn), will grow much faster under increased temperatures (Werkman & Callaghan, 1999). This resulted in a reduction in *C. vulgaris* cover due to increased shading by *P. aquilinum* (Werkman & Callaghan, 1999). Dawes *et al*. (2011) suggest that increased evaporation potential under higher temperatures may also have contributed to species loss in an alpine dwarf shrub community. However, van Meeteren *et al*. (2008) reported lower phosphorus mineralisation and reduced foliar

phosphorus concentrations in a Dutch heathland subjected to simulated climate warming. As heathland systems are generally nutrient deficient, and growth is either nitrogen or phosphorus limited, van Meerteren *et al*.'s (2008) observations suggest that a reduction in nutrient availability due to climate warming might retard graminoid growth and reduce encroachment (Peñuelas *et al*., 2004; van Meeteren *et al*., 2008).

These changes in nutrient availability are confounded by changes in atmospheric CO_2 concentrations and precipitation. Andresen *et al*. (2010a; 2010b) suggest that interactions between temperature, precipitation and CO_2 concentrations are complex, site and species specific, and vary seasonally. Larsen *et al*. (2011) suggest that increased drought stress under a climate change scenario will reduce N cycling and plant growth to a greater extent than increases in temperature or CO_2 concentration. Consequently this would complicate the prediction of climate warming effects on heathland systems in mainland Britain, which are expected to experience increased CO_2 concentrations and a change in the distribution of precipitation under a climate change scenario (IPCC, 2007). Additionally, it should be noted that many of these studies are based on modelled ground-level atmospheric ambient temperatures, rather than prediction of soil level temperatures. As many ecological processes that affect plants occur in, or at the surface of the soil, rather than high in the atmosphere, this may lead to errors in the predictions of the impact of climate warming on heathlands as microclimatic variations in temperature have not been taken into account (Graae *et al*., 2011).

Jeffers *et al*. (2011) indicated that past increases in temperature have facilitated the invasion of *Betula* spp. into heathland systems due to the greater temperature-dependent growth response of *Betula* spp. when compared to ericaceous species. Peñuelas *et al.* (2004) reported increases in

L. suturalis herbivory following temperature increases, possibly due to enhanced insect metabolism or decreased plant nutritional value necessitating greater consumption. Therefore there are many potential effects of climate warming on British heathlands and these will interact with other environmental changes, such as increased atmospheric CO_2 and drought frequency.

1.6 Research aims and objectives

This project focused on the remaining heathland fragments in the East Midlands region of Britain, which encompasses Nottinghamshire, Leicestershire, Lincolnshire, Derbyshire and Rutland, and sought to place them in a national perspective. Most heathland areas are considered to be of high conservation value and are afforded the protection of nature reserve or SSSI status. This project aimed to investigate the extent to which these remaining heathlands have been modified ecologically by over a century of elevated N deposition. Controlled experiments suggest that N enrichment drives plant community changes, habitat fragmentation, and ultimately heathland loss. These studies frequently observe the impact of short-term, ecologically improbable large artificial additions of N on soil nutrient status, plant growth, nutrient uptake and community change. The objective of this research was to examine the extent to which these predicted impacts of N enrichment have occurred in a region with a long history of N pollution and further, how these potentially detrimental impacts have been modified by management practices.

The aims of this project were:

(i) To measure the extent to which the fertility of heathland soils has been modified by nitrogen pollution, including NH_3 emissions.

(ii) To determine the effect of fragmentation, N enrichment, geographical location and local site characteristics, including management, on higher plant, bryophyte and lichen composition and diversity.

(iii) To study response to simulated warming of *Calluna vulgaris* populations sampled along a latitudinal gradient in Europe.

This study provides a valuable insight into how heathland soil fertility and vegetation composition has been affected by over a century of nitrogen pollution. This investigation will complement previous research which has linked N enrichment to increased soil fertility and vegetation composition change, but has done far less to describe how these responses have transferred to the field situation. The current study will inform heathland management policy in order to help conserve the remaining heathland patches in Britain.

1.7 Outreach and the OPAL Project

This project was part of the Open Air Laboratories (OPAL) Project East Midlands region. OPAL was a nationwide initiative which intended to encourage members of the public, particularly school students, to explore, study and enjoy their local environment (www.OPALexplorenature.org). As part of the initiative, the East Midlands region team of Community Science Officers used data from this study to develop resources to distribute to schools and youth groups in the region, including those in hard-to-reach areas of the community. The resources included raw or partially analysed data for use in GCSE and A-Level classes, and a field guide for use in outreach events (Appendix 1). The field guide was based on plant, bryophyte and lichen species compiled during this study.

2

Site Selection and Description

2.1 Introduction

This project focused on the remaining heathland fragments in the East Midlands region of England. This region is in the bottom third of annual rainfall levels in the British Isles (R. Smith, CEH, Edinburgh, Pers. Comm). Additional heathlands were also selected across mainland Britain to provide sites subjected to a broader range of N deposition than was represented in the East Midlands alone. At each site plant community composition was described and soil fertility was inferred by means of a bioassay in which the yield of *Calluna vulgaris* was used as the indicator, and relationships sought between plant response and anthropogenic N enrichment. Soil samples were collected at all sites to furnish bioassays to assess heathland soil fertility (Chapter 3) and for phosphomonoesterase activity measurements (Chapter 4). The mitigating effect of management practices was also investigated.

2.2 Site selection

2.2.1 East Midlands sites

The first objective was to locate lowland heathlands within the East Midlands region of England. Lowland heathland was defined as vegetation comprising >25% *C. vulgaris* cover and located <300 m above sea level. Between October and December 2008 all lowland heathlands that were located within the East Midlands were visited and assessed for suitability for inclusion in this study (e.g. did a site qualify as heathland). Sites from a previous survey by

Hyvärinen & Crittenden (1998) were included. County Wildlife Trusts and regional Natural England offices were also consulted, and local knowledge in the School of Biology, The University of Nottingham, was utilised. This resulted in a total of 16 sites being identified, of which 11 were used in the study (Table 2.1). Five sites were rejected due to either low *C. vulgaris* cover (<25%), high water table (e.g. sometimes indicated by the presence of *Sphagnum* spp.), negligible organic soil layer or access refusal by landowners (Appendix 2).

2.2.2 National sites

The second objective was to place the East Midland sites in a national context by comparing these sites with others from physiographically similar areas with lower and higher N deposition values than those for the East Midlands (Figures 2.1 and 2.2). Modelled values of N deposition at the heathland sites were obtained from a 5 x 5 km gridded data set provided by R.I. Smith for 2006 (Pers. Comm., CEH, Edinburgh). These data sets consisted of the following values: annual mean wet deposited inorganic N (N_W = NO_3^- - N + NH_4^+ - N), dry deposited inorganic oxidised N (N_{DO} = NO_2 - N + NO_3^- - N + HNO_3 - N) and dry deposited inorganic reduced N (N_{DR} = NH_3 - N + NH_4^+ - N).The data were derived using an atmospheric deposition model parameterised for moorland terrain (Smith & Fowler, 2001). The model uses a simulated rainfall field for the UK generated by the UK Meteorological Office (data from which were also available for this study), and N deposition measurements from the UK Acid Deposition Monitoring Network (AEA Technology PLC, Didcot, UK). Data for 2006 were used to select heathland sites. In 2010 mean data were obtained for the period 2000 - 2008, and so all subsequent analyses (as opposed to site selection) were conducted using mean N deposition values for this period.

Table 2.1 Details of sites included in this study. Modelled values of inorganic N deposition and rainfall were provided by R.I. Smith (CEH, Edinburgh). Sites were numbered using the 2006 modelled N deposition data used in site selection.

Site Number	Site Name	Responsible Body/Organisation/ Individual	Grid Ref	5 x 5 km square	Modelled Rainfall (mm y^{-1})	Modelled Wet Inorganic N Deposition (kg ha^{-1} y^{-1})†		Site Area (ha)
						2006 i	2000 - 08 ii #	
1	Heathmount North	Balnagown Estate	NH765792	275E 875N	793	2.00	1.86	97.09
2	Hunting Hill	Mr. G MacLeod, Tenant Farmer	NH812805	280E 880N	836	2.40	2.11	108.00
3	Lundy Island	The Lundy Company Ltd	SS137463	210E 145N	774	4.40	4.17	16.35
4	Goonhilly Downs	Natural England	SW729198	170E 015N	636	4.90	5.16	1101.13
5	Coverack	Natural England	SW781199	175E 015N	686	5.50	5.63	11.81
6	Ashdown Forest	The Conservators of Ashdown Forest	TQ455285	545E 125N	785	5.80	7.13	320.64
7	Hothfield Common	Kent Wildlife Trust	TQ967457	595E 145N	720	6.10	6.97	7.57
8	Horsell Common	Horsell Common Preservation Society	TQ005607	500E 160N	747	6.40	6.31	1.38
9	* Woodhall Spa Golf Club	Woodhall Spa National Golf Centre	TF210643	520E 360N	549	6.70	6.67	3.21

10	* Kirkby Moor	Lincolnshire Wildlife Trust	TF215632	520E 360N	549	6.70	6.67	0.61
11	Skipwith Common	Escrick Park Estate	SE660374	465E 435N	617	6.90	6.77	22.26
12	* Clumber Park	The National Trust	SK615710	460E 375N	594	7.20	7.37	2.67
13	* Scotton Common	Lincolnshire Wildlife Trust	SK873985	485E 395N	607	7.50	7.37	6.88
14	* Walesby Forest	Walesby Outdoor Activity Centre	SK668702	465E 370N	649	8.10	8.17	2.21
15	* Ulverscroft	Leicestershire and Rutland Wildlife Trust	SK491126	445E 310N	684	8.40	8.30	0.22
16	* Budby Heath	Thoresby Estate & Ministry of Defence	SK615692	460E 365N	664	8.80	8.91	134.54
17 a/b	* Beacon Hill I/II	Leicestershire County Council	SK509148	450E 310N	761	9.10	9.11	0.52/0.09
18	Woolley Moor	Wakefield Council	SE323141	430E 410N	714	9.80	9.93	4.38
19	* Sherwood Forest Golf Club	Sherwood Forest Golf Club	SK585615	455E 360N	755	9.90	9.88	11.95
20	*Oak Tree Heath	Mansfield District Council	SK568603	455E 360N	755	9.90	9.88	5.46
21	* Vicar Water Country Park	Newark and Sherwood District Council	SK585623	455E 360N	755	9.90	9.88	11.40
22	Brizlee Wood	Natural England	NU145138	410E 610N	815	10.80	9.51	349.28

23	Thimbleby Moor	Thimbleby Estate	SE470955	445E 495N	831	11.00	9.72	5542.78
24	Litcham Common	Norfolk County Council	TF888175	585E 315N	725	11.40	10.42	1.10
25	Holt Lowes	Trustees of Holt Lowes	TG088376	605E 335N	712	12.10	10.90	11.00

† N; NO_3^- + NH_4^+
* East Midlands sites
# (i) used for initial site selection, (ii) used for all subsequent analyses.

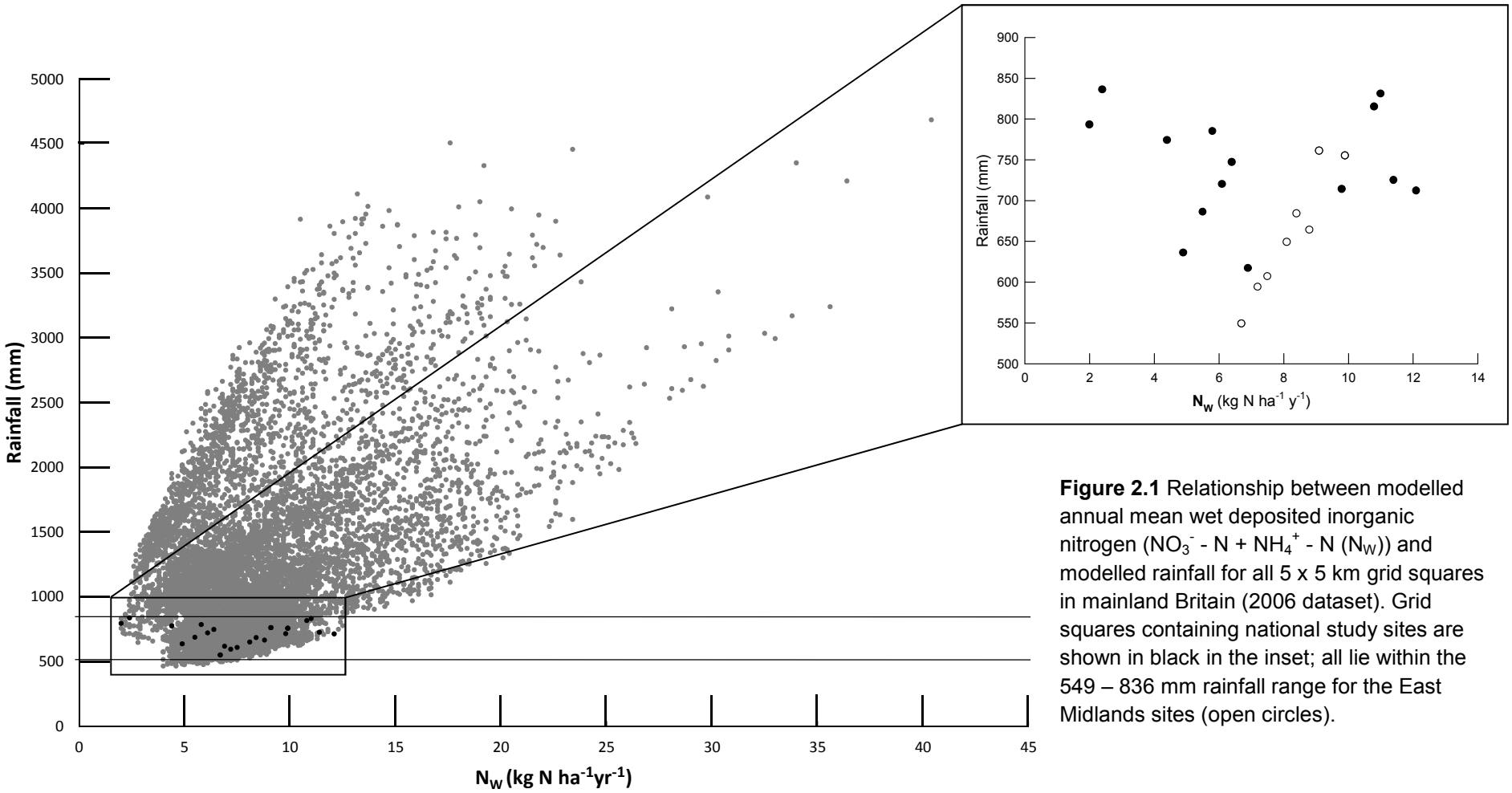


Figure 2.1 Relationship between modelled annual mean wet deposited inorganic nitrogen (NO_3^- - N + NH_4^+ - N (N_W)) and modelled rainfall for all 5 x 5 km grid squares in mainland Britain (2006 dataset). Grid squares containing national study sites are shown in black in the inset; all lie within the 549 – 836 mm rainfall range for the East Midlands sites (open circles).

Figure 2.2 Location of heathlands in England and Scotland used in the present investigation. Site numbers correspond to those presented in Table 2.1.

Annual rainfall in the East Midlands is amongst the lowest in mainland Britain and ranges between 549 – 836 mm y^{-1}. National sites therefore were selected from locations with rainfall values within this range (Figure 2.1 and 2.3) and using N_W values from the 5 x 5 km gridded data set. N_W values were used in site selection in preference to total N deposition because modelled N_W data are based on measured values of wet deposition (NO_3^- + NH_4^+), whereas total N deposition includes dry N deposition data which are derived from transfer models and hence modelled interpolated values of N_{DO} and N_{DR} contain large uncertainties (Magnani *et al*., 2007). Between October 2008 and March 2009 sites were identified using the same methods as for the East Midlands. Further sites were located using www.natureonthemap.naturalengland .org.uk, an inventory of habitats within mainland Britain compiled by Natural England. All sites were visited and assessed for suitability. Twenty-eight additional sites were located, of which 14 were selected for use. Thirteen sites were rejected for the reasons described above, and one site was rejected due to safety concerns.

2.3 Determination of site area

The area of heathland at each site was determined by walking around the site boundary with a handheld GPS unit (Garmin 400t, Garmin Ltd, Kansas City, USA) while recording waypoints at regular intervals. The site boundary was defined when *C. vulgaris* (or other dominant ericoid) was no longer present. If the site was too large to measure on foot then the area was determined using digitised Ordnance Survey maps (EDINA, Edinburgh, UK) and aerial photographs (Google Inc., California, USA) in ArcMap v9.3.1 (ESRI Inc, California, USA). The spatial accuracy of aerial photographs depends greatly on the altitude and angle of the camera used to capture the image. Photographs therefore were geo-referenced using overlain digitised Ordnance

Survey maps, and identifiable landmarks used to calibrate the area covered by the image. The heathland area for the Ashdown Forest site was provided as .shp files by C. Marrable at the Ashdown Forest Centre. All areas were obtained using the Area Calculation function in ArcMap v9.3.1.

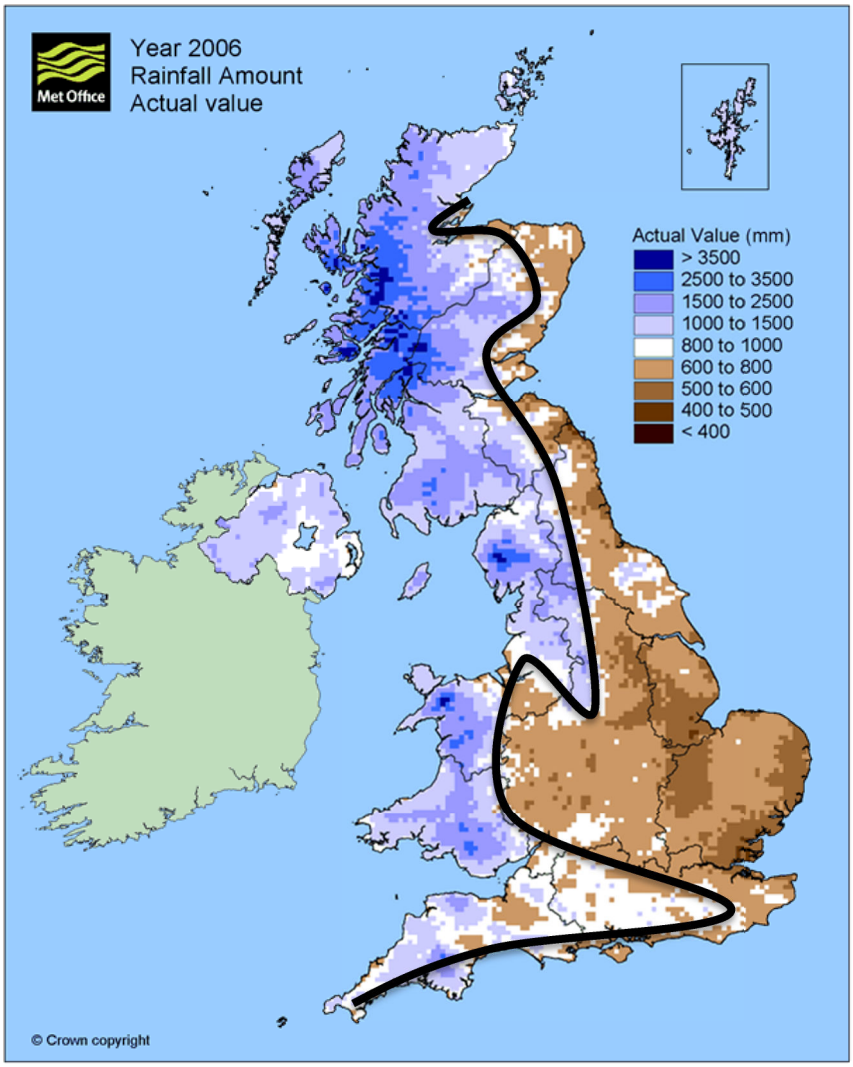


Figure 2.3 A map of interpolated total annual rainfall (mm) in the United Kingdom during 2006 (MET Office). The line illustrates the boundary between regions of mainland Britain with rainfall values within the range of the East Midlands region (549 - 836 mm y^{-1}), and those outside that range. Within this area there was further selection for areas below 300 m altitude and for differing levels of N deposition (N_W).

2.4 Vegetation Classification and Underlying Geology

Plant community types for all sites were classified into the phyto-sociological units of the British Vegetation Classification (Rodwell 1991 and 1992) using the plant composition data yielded by the vegetation survey (Chapter 6). Communities consisted of heathland (NVC H1, 1e, 2c, 6d, 8, 9, 9c, 9e, 10, 11), mire (M15d, 25a) and acid grassland (U2a, 2b) vegetation types (Table 2.2). Figure 2.4 illustrates differences in vegetation community types at a selection of sites across the N_W deposition gradient. Figure 2.4a illustrates a site with a low N_W deposition of 2.40 kg N ha^{-1} y^{-1}, but in close proximity to a cattle farm, with vegetation dominated by *C. vulgaris*. Figure 2.4b shows a large area of heathland in SW England with N_W deposition of 4.90 kg N ha^{-1} y^{-1} dominated by *Erica vagans* and *Ulex europaeus*, surrounded by woodland. Figures 2.4c to 2.4f illustrate the differences in vegetation composition at four sites in the East Midlands. Figure 2.4c and 2.4f show heathlands subject to N_W deposition of 6.70 and 9.90 kg N ha^{-1} y^{-1} respectively, that are located on golf courses, and surrounded by woodland and heavily managed grassland communities. Figures 2.4d and 2.4e, on the other hand, show two small sites with N_W deposition of 7.2 and 8.4 kg N ha^{-1} y^{-1} respectively, in which *Pteridium aquilinum* and grass species, such as *Deschampsia flexuosa,* are frequent. Finally, figures 2.4g and 2.4h illustrate two heavily managed large sites with relatively high N_W deposition values (10.80 and 12.10 kg N ha^{-1} y^{-1}), but which are dominated by *C. vulgaris*, with limited invasion by graminoids.

Bedrock geology and superficial deposit information for all sites was obtained from 1:50000 scale data from the OpenGeoscience Geology of Britain online resource provided by the British Geological Survey (Table 2.2; http://maps.bgs.ac.uk/geologyviewer_google/googleviewer.html).

A

B

Figure 2.4 Examples of vegetation composition at (A) Hunting Hill (NH812805) N_W = 2.4 kg ha^{-1} y^{-1}, *Calluna vulgaris* – *Deschampsia flexuosa* heath with a species poor sub-community (NVC H9c), and (B) Goonhilly Downs (SW729198) N_W = 4.9 kg ha^{-1} y^{-1}, *Erica vagans* – *Ulex europaeus* heath with a *Molinia caerulea* sub-community (NVC H6d).

C

D

Figure 2.4 Examples of vegetation composition at (C) Woodhall Spa (TF210643) N_W = 6.7 kg ha^{-1} y^{-1}, *Calluna vulgaris – Deschampsia flexuosa* heath (NVC H9), and (D) Clumber Park (SK615710) N_W = 7.2 kg ha^{-1} y^{-1}, *Deschampsia flexuosa* grassland with a *Festuca ovina – Agrostis capillaries* sub-community (NVC U2a).

E

F

Figure 2.4 Examples of vegetation composition at (E) Ulverscroft (SK491126) N_W = 8.4 kg ha^{-1} y^{-1}, *Deschampsia flexuosa* grassland with a *Festuca ovina – Agrostis capillaris* sub-community (NVC U2a), and (F) Sherwood Forest Golf Club (SK585615) N_W = 9.9 kg ha^{-1} y^{-1}, *Calluna vulgaris – Deschampsia flexuosa* heath with a *Galium saxatile* sub-community (NVC H9).

G

H

Figure 2.4 Examples of vegetation composition at (G) Brizlee Wood (NU145138) N_W = 10.8 kg ha^{-1} y^{-1}, *Calluna vulgaris – Deschampsia flexuosa* heath with a species-poor sub-community (NVC H9c), and (H) Holt Lowes (TG088376) N_W = 12.1 kg ha^{-1} y^{-1}, *Calluna vulgaris – Ulex gallii* heath (NVC H8).

Table 2.2 Description of plant community types, bedrock geology type and superficial deposits at all heathland sites in the current study. Plant community types were classified using Rodwell (1991) and Rodwell (1992). Bedrock geology type and superficial deposits were obtained from 1:50000 scale data from the OpenGeoscience Geology of Britain online resource (http://maps.bgs.ac.uk/geologyviewer_google/googleviewer.html - 14/11/11).

Site Number	Site Name	* NVC Classification	Vegetation Community Type	Bedrock Geology	Superficial Deposits
1	Heathmount North	H11	*Calluna vulgaris* - *Carex arenaria* heath	Raddery Sandstone formation - pebbly (gravelly) sandstone	Till, Devensian, Diamicton
2	Hunting Hill	H9c	*Calluna vulgaris* - *Erica cinerea* heath with a species-poor sub-community	Raddery Sandstone formation - pebbly (gravelly) sandstone	Till, Devensian, Diamicton
3	Lundy Island	H8	*Calluna vulgaris* - *Ulex gallii* heath	Lundy Island Intrusion	None recorded
4	Goonhilly Downs	H6d	*Erica vagans* - *Ulex europaeus* heath with a *Molinia caerulea* sub-community	Lizard Complex - Peridotite and serpentine	None recorded
5	Coverack	H6d	*Erica vagans* - *Ulex europaeus* heath with a *Molinia caerulea* sub-community	Unnamed igneous intrusion, Devonian - Gabbro	None recorded
6	Ashdown Forest	H2c	*Calluna vulgaris* - *Ulex minor* heath with a *Molinia caerulea* sub-community	Ashdown formation - Sandstone and siltstone, interbedded	None recorded
7	Hothfield Common	H9e	*Calluna vulgaris* - *Deschampsia flexuosa* heath with a *Molina caerulea* sub-community	Sandgate formation - sandstone, siltstone and mudstone. Some Folkestone formation - sandstone	Some alluvium - silty, peaty, sandy clay
8	Horsell Common	H9e	*Calluna vulgaris* - *Deschampsia flexuosa* heath with a *Molina caerulea* sub-community	Bagshot formation - sand	None recorded
9	Woodhall Spa Golf Club	H9	*Calluna vulgaris* - *Deschampsia flexuosa* heath	Ampthill Clay formation - mudstone	Glaciofluvial sheet deposits, mid-Pleistocene sand and gravel

10	Kirkby Moor	H9c	*Calluna vulgaris - Erica cinerea* heath with a species-poor sub-community	Ampthill Clay formation - mudstone	Glaciofluvial sheet deposits, mid-Pleistocene sand and gravel
11	Skipwith Common	M25a	*Molinia caerulea - Potentilla erecta* mire with an *Erica tetralix* sub-community	Sherwood Sandstone group - sandstone	Skipwith Sand member - clayey, gravelly sand
12	Clumber Park	U2a	*Deschampsia flexuosa* grassland with a *Festuca ovina - Agrostis capillaris* sub-community	Nottingham Castle Sandstone formation - pebbly (gravelly) sandstone	None recorded
13	Scotton Common	M15d	*Scripus cespitosus - Erica tetralix* wet heath with a *Vaccinium myrtillus* sub-community	Scunthorpe Mudstone formation - mudstone and limestone, interbedded	Blown sand 1 - sand
14	Walesby Forest	H9	*Calluna vulgaris - Deschampsia flexuosa* heath	Nottingham Castle Sandstone formation - pebbly (gravelly) sandstone	None recorded
15	Ulverscroft	U2a	*Deschampsia flexuosa* grassland with a *Festuca ovina - Agrostis capillaris* sub-community	Gunthorpe member mudstone. Some North Charnwood Diorite - diorite	Head - clay, silt, sand and gravel
16	Budby Heath	U2a	*Deschampsia flexuosa* grassland with a *Festuca ovina - Agrostis capillaris* sub-community	Nottingham Castle Sandstone formation - pebbly (gravelly) sandstone	None recorded
17a	Beacon Hill I	H10	*Calluna vulgaris - Erica cinerea* heath	Beacon Hill formation - Volcaniclastic siltstone	Head - clay, silt, sand and gravel
17b	Beacon Hill II	U2b	*Deschampsia flexuosa* grassland with a *Vaccinium myrtillus* sub-community	Beacon Hill formation - Volcaniclastic siltstone	Head - clay, silt, sand and gravel
18	Woolley Moor	H9	*Calluna vulgaris - Deschampsia flexuosa* heath	Woolley Edge Rock - sandstone. Some Pennine Middle Coal Measures formation - mudstone, siltstone and sandstone	None recorded
19	Sherwood Forest	H9	*Calluna vulgaris - Deschampsia*	Nottingham Castle Sandstone formation -	None recorded

	Golf Club		*flexuosa* heath	pebbly (gravelly) sandstone	
20	Oak Tree Heath	H9	*Calluna vulgaris - Deschampsia flexuosa* heath	Nottingham Castle Sandstone formation - pebbly (gravelly) sandstone	None recorded
21	Vicar Water Country Park	H1	*Calluna vulgaris - Festuca ovina* heath	Nottingham Castle Sandstone formation - pebbly (gravelly) sandstone	None recorded
22	Brizlee Wood	H9c	*Calluna vulgaris - Erica cinerea* heath with a species-poor sub-community	Fell Sandstone formation - sandstone	Some till, Devensian - Diamicton
23	Thimbleby Moor	H1e	*Calluna vulgaris - Festuca ovina* heath with a species-poor sub-community	A mix of: Moor Grit member - sandstone. Scarborough formation - mudstone. Crinoid Grit member - sandstone. BrandsbyRoadstone member - interbedded sandstone and [subequal/subordinate] limestone. Cloughton formation sandstone	None recorded
24	Litcham Common	M25a	*Molinia caerulea - Potentilla erecta* mire with an *Erica tetralix* sub-community	A mix of: Lewes Nodular Chalk formation. Seaford Chalk formation. Newhaven Chalk formation. Culvar Chalk formation	River terrace deposits (undifferentiated) - sand and gravel
25	Holt Lowes	H8	*Calluna vulgaris - Ulex gallii* heath	A mix of: Lewes Nodular Chalk formation. Seaford Chalk formation. Newhaven Chalk formation. Culvar Chalk formation	Briton's Lane Sand and Gravel member - sand and gravel

* NVCs were determined using the Tablefit programme (M. Hill, CEH Oxfordshire)

2.5 Site management

Landowners and managers of all sites were contacted and asked a series of standardised questions to obtain information about the management strategies at each site. The questions asked were:

1. Is there any management on the heathland patches?
2. Are there any particular management interests? (e.g. rare plant species, ground nesting birds, insects, lichens, game shooting)
3. How often is the heathland managed on average?
4. Is the management patchy across the heathland? (e.g. small areas mown on rotation)
5. When was the site last mowed/grazed/sod-cut/burnt?
6. Has heather been added recently? (e.g. as seed or seedlings)
7. Have any trees/bracken/rhododendron/gorse bushes been removed recently?
8. Was the site developed, or has heathland always been present? (e.g. developed from a seed bank by scraping or by spreading seeds)
9. Have there been any other improvements to the heathland? (e.g. drainage works)
10. Is there anything else that you would like to add?

The application of management techniques varied substantially between sites, and varied spatially and temporally within sites. The extent of historical records of the management applied at each site also varied considerably. Table 2.3 summarises the management strategies of the heathland sites in this study. All sites have been managed in the past, and all but one site were subject to management within five years of the study period. Eighteen sites were grazed, burning was varied spatially within sites and records were unreliable, *P. aquilinum* and tree removal occurred sporadically. Sod was removed at two sites within 15 y of the study period.

Table 2.3 Summary of management techniques used at the 25 study sites.

Site Number	Site Name	Site Contact	Management Summary
1	Heathmount North	Mr. R Mackenzie Tenant Farmer	No extensive management at this site. May have been burned in the past, but the extent and dates are not known.
2	Hunting Hill	Mr. G MacLeod Tenant Farmer	Some patchy controlled burning since 1990. Small areas burnt during 2009, after initial soil collection. Very low-density sheep grazing since 2002.
3	Lundy Island	Ms. S Wheatley Assistant Warden	Unmanaged grazing by rabbits, ponies, deer, Soay sheep and goats at low-densities. *P.aquilinum* removed by bruising every July.
4	Goonhilly Downs	Mr. R Lawman Natural England	Grazed by ponies and cattle. Livestock rotated around the site annually. Patches of heathland approximately 2 ha in area burnt every ten years. *Pinus* spp. removed when necessary.
5	Coverack	Mr. R Lawman Natural England	Grazed by ponies and cattle. Livestock rotated around the site annually. Patches of heathland approximately 2 ha in area are burnt every ten years. *Pinus* spp. are removed when necessary.
6	Ashdown Forest	Mr. C Marrable Ashdown Forest Centre	Burnt and mown to maintain heterogeneity. Frequency of applications vary. Management for conservation began in 1984. *P.aquilinum* and *Ulex* spp. removed as necessary.
7	Hothfield Common	Mr. I Rickards Kent Wildlife Trust	Since 2006 moderate density grazing by sheep and cattle, with densities increased during May-October annually. Areas of woodland and scrub are removed as necessary. *P.aquilinum* sprayed annually with Asulox. No burning since 2000.
8	Horsell Common	Mr. P Rimmer Estate Manager	Trees and scrub is removed when necessary. There is no other extensive management.
9	Woodhall Spa Golf Club	Mr. S Rhodes Course Manager	Mown in 2007. Entire site burnt in 1960. No other management
10	Kirkby Moor	Mr. D Bromwich	From 1970 to 1998 very limited *P.aquilinum* removal. Post 1998 *P.aquilinum* has

		Lincolnshire Wildlife Trust	been regularly treated with Asulox. Very low-density grazing by sheep present 2000.
11	Skipwith Common	Ms. T Fawcett Escrick Park Estate	Grazing by sheep, cattle and ponies at moderate densities applied in a bi-annual rotation. Stocking densities vary throughout the year, but were extremely high during 2001 due to the foot and mouth outbreak. Small area burnt during 2005. *Betula* spp. removed when necessary.
12	Clumber Park	Mr. P Bowell National Trust	Trees and scrub removed as necessary. Some areas grazed at low densities by sheep. Some *C. vulgaris* seed added to promote heathland regeneration after site damage when used as a car park before 2000.
13	Scotton Common	Mr. D Bromwich Lincolnshire Wildlife Trust	Grazing by sheep and cattle occurred at varying stocking densities since 1990. *Betula* spp. removed when necessary, with a significant period of removal during the 1990s to promote heathland restoration.
14	Walesby Forest	Mr. I Sinclaire Walesby Forest Centre	May have been mown, but no record of management.
15	Ulverscroft	Mr. N Pilcher Leicestershire & Rutland Wildlife Trust	During the late 1990s *C. vulgaris* seed was spread in a small area to promote heathland restoration. Grazing at low to moderate densities with long-horned cattle introduced in 2005. *P.aquilinum, Ulex* spp., and *Rubus fruticosus* agg. removed when necessary.
16	Budby Heath	Mr. T Hill Thoresby Estate	Severely damaged during World War II due to use as an MOD training ground. Conservation began post-1980. Grazing by sheep and cattle at moderate densities on-going since 1980s. Livestock rotated annually. Scrub and tree clearance and *P.aquilinum* removal by spraying with Asulox occurs annually in the summer.
17a	Beacon Hill I	Mr. M Moffatt Leicestershire County Council	The organic soil layer was removed in 1995 to reduce soil fertility and promote heathland regeneration from the existing seed-bank. The site is grazed by sheep at various stocking densities. Annually, during April to November the entire site is mown. *Betula* spp., *P. aquilinum* and *Ulex* spp. are removed when necessary.
17b	Beacon Hill II	Mr. M Moffatt Leicestershire County	Grazed throughout the year by cattle at very low densities. *P.aquilinum* and scrub removed when necessary.

		Council	
18	Woolley Moor	Ms. S Worrall Wakefield Council	*Calluna vulgaris* cut in small patches annually to create a heterogeneous age structure. Scrub removed annually between October and March. *Pteridium aquilinum* is sprayed with Asulox annually in August. An uncontrolled fire severely damaged part of the heathland in 2009.
19	Sherwood Forest Golf Club	Mr. G Mason Club Manager	Grazing with sheep at low densities occurs throughout the year. Trees and scrub removed when necessary.
20	Oak Tree Heath	Ms. F Washbrook Mansfield District Council	Annually patches of *C. vulgaris* mown to create a heterogeneous age structure. Trees and scrub removed when necessary. *Pteridium aquilinum* is sprayed with Asulox annually during the summer. Accidental fires occur sporadically.
21	Vicar Water Country Park	Mr. K Hatfield Site Warden	Developed on an industrial spoil heap during the 1970s by the addition of *C. vulgaris* seed. Further heathland areas developed between 1993 and 2000. Grazing by sheep at low stocking densities used throughout the year.
22	Brizlee Wood	Mr. S Pullan Natural England	Grazing by sheep at low stocking densities present throughout the year. Small areas (approximately 15% of the site) burnt in rotation annually.
23	Thimbleby Moor	Mr. M Owen Natural England	*Betula* spp. and *P. aquilinum* removed when necessary. Small areas burnt in rotation annually. Each area is burnt once every ten years.
24	Litcham Common	Ms. S Cureton Norfolk County Council	Grazed with low stocking densities of ponies throughout the year. Scrub removed annually during the winter. *Pteridium aquilinum* removed during the summer.
25	Holt Lowes	Mr. S Harrap Trustee of Holt Lowes	Grazing by sheep and cattle at low stocking densities applied throughout the year. During the mid-1990s extensive cutting and bulldozing occurred to remove *Ulex* spp. and woodland. Uncontrolled fires occurred during 1984 - 1985, causing significant damage to the entire site.

3

An Assessment of Lowland Heathland Soil Fertility Across an N_W Deposition Gradient

3.1 Introduction

A primary objective of this research was to discover the extent to which the fertility of heathlands in an industrial region of lowland England has been modified by anthropogenic environmental drivers, most notably by modern N deposition loads. The impact of N enrichment on soil fertility and plant species composition has been demonstrated, for example, in grasslands by Stevens *et al*. (2004), in woodlands by Bobbink *et al*. (2008) and Kirby *et al*. (2005), and in heathlands by Maskell *et al*. (2010) and Power *et al*. (1995). Plant species composition has been strongly linked to soil N availability in a variety of temperate habitats (Maskell *et al.*, 2010; McClean *et al*., 2011). Nitrogen limited systems, such as heathlands, are particularly susceptible to N enrichment (Lee, 1998; Lee & Caporn, 1998), which has been shown to result in a change from heathland into grassland (Heil & Diemont, 1983) or heathland into woodland (Hester *et al*., 1991). Despite widespread conservation efforts, the average area of heathlands in Britain has decreased in recent decades (Rose *et al*., 2000). The surviving remnant heathlands are all considered of high conservation value with most being designated as Sites of Special Scientific Interest, National Nature Reserves, or Local Nature Reserves. The present assessment was intended to identify levels of threat.

There is abundant evidence from controlled field manipulation experiments that N enrichment can increase the growth rate of heathland plants and drive ecosystem change. Aerts (1989) acknowledged that ericaceous species, such

as *Calluna vulgaris* and *Erica* spp. show increased growth under N and P enrichment. Aerts & Berendse (1988), however, demonstrated that the graminoid *Molinia caerulea* showed a much greater growth response to N enrichment than *Erica tetralix,* and so was able to outcompete the slower growing ericoid, thus ultimately resulting in heathland loss. Fragmentation also has detrimental effects on habitats and might influence the rate of nutrient ingress when nutrient poor habitats remain in a well fertilised agricultural landscape. Furthermore, heathland management practices, some of which are carried out specifically to reduce fertility, might be expected to modify pollutant impacts.

In order to discover the extent to which heathland fertility might have been changed by these environmental pressures and management counter-measures, a bioassay was undertaken to quantify relationships between soil fertility and N deposition, heathland patch size and management regimes. Using plant growth as a bioassay system to investigate the impact of pollutants on soil fertility and plant tissue chemistry has proved successful in past studies (Feder, 1978). For example Laperche *et al*. (1997) used *Sorghum bicolor* in a bioassay to assess lead uptake and how this was modified by various compounds. Zebarth *et al*. (2005) used potato crops as a bioassay system to assess the availability of N, P and K after artificial N applications.

This chapter reports investigations into the relationship between modelled N_W values at 25 low-rainfall lowland heathland sites in Britain in the range 1.85 - 10.90 kg N ha^{-1} y^{-1} and *C. vulgaris* S_W, $[N]_{shoot}$, $[P]_{shoot}$ and $([N]:[P])_{shoot}$. The extent to which these plant variables have been modified by management practices was also studied. The effects of geographical location, rainfall and heathland patch size on *C. vulgaris* growth were also investigated.

3.2 Materials and Methods

3.2.1 Soil collection and pre-treatment

Ten replicate soil samples were collected at the 25 sites described in Chapter 2 between 2 April and 8 May 2009. Replicate samples were taken at spots at least 10 m apart. At each spot a 10 x 10 cm block of organic top soil to a depth of 5 cm was removed from amongst *C. vulgaris* bushes and passed through a 3 mm sieve. The residual debris was replaced in the ground. Soil samples, each approx. 100-200 ml in volume, were then stored at *c*. 5°C. Maximum storage time was three weeks.

3.2.2 Plant bioassay

Calluna vulgaris seeds were collected from Beacon Hill, Leicestershire, in November 2007, air dried and stored at *c.* 5°C. In April 2009 seeds were germinated on agar at 18°C for one month. Bags 8 x 7 cm were constructed from 30 µm woven nylon mesh (Clarcor, Lockertex, NY/MO/30/19). Each bag was filled with *c*. 85 ml of moderately compressed wet soil, placed centrally in a 160 ml plant pot, and the surrounding space (*c*. 75 ml) filled with water-washed horticultural silver sand (Figure 3.1). The arrangement of soil sample surrounded by sand ensured that voids did not appear in the soil during watering and that the soil remained uniformly wet. Two *c.* 40-day-old *C. vulgaris* seedlings were transplanted into the soil in each pot and the pots placed in a growth room on a 14 h light (18°C) / 10 h dark (12°C) programme.

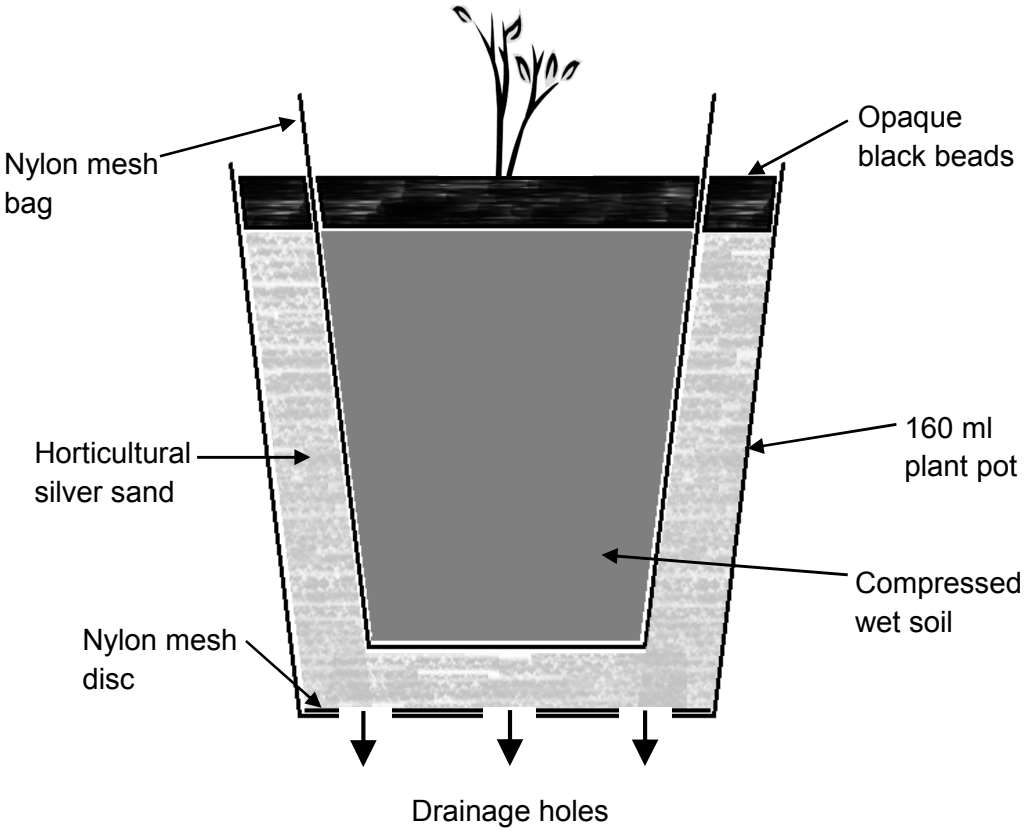


Figure 3.1 Illustration of plant pot apparatus used in the bioassay.

Relative humidity was maintained at approximately 80 % using a Faran HR-15 centrifugal atomising humidifier (Faran Industrial Co., Korea), and a photosynthetic photon fluence rate of 274 μmol m^{-2} s^{-1} (over the waveband 200-400 nm) was maintained at soil level throughout the light period from a bank of 48 fluorescent tubes. When the seedlings were sufficiently large, opaque black beads were placed on top of the sand to suppress seed germination and growth of algae and bryophytes. Fifteen pots were grouped in blocks in seed trays which were rotated within the growth room at weekly intervals to control for light and temperature gradients. Each seed tray contained soils from 15 different locations and sites were distributed in a regular and sequential manner throughout the population of 257 pots (Figure 3.2). Deionised water was added to the sand to excess (i.e. to generate through flow) at intervals of two days.

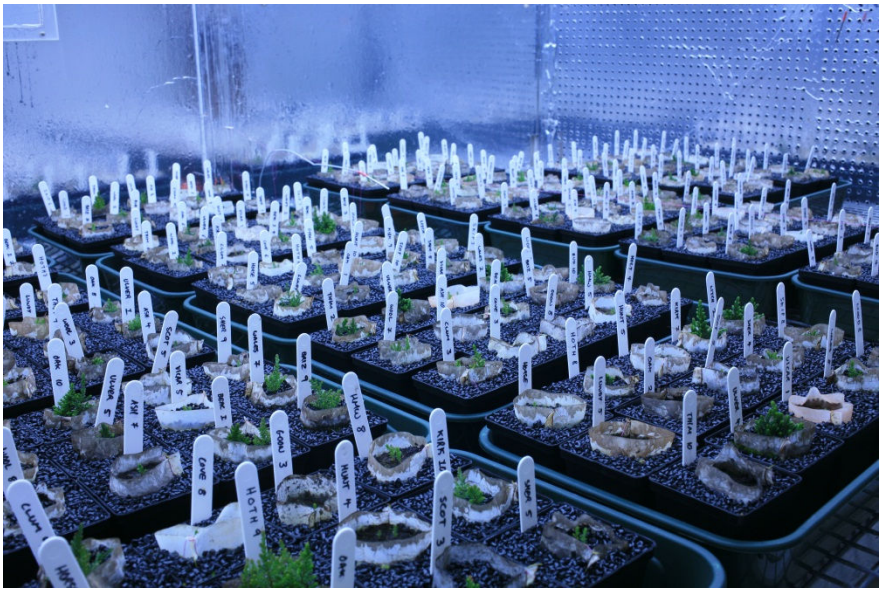


Figure 3.2 *Calluna vulgaris* seedlings in controlled environment growth room.

Plants were harvested after 18 weeks of growth. Quantitative separation of fine roots from the soil was found to be problematic due to the large quantities of adhering organic debris. Thus shoots were detached from roots, washed free of soil, oven dried for 24 h at *c.* 80°C, and weighed.

3.2.3 Examination for mycorrhizal infection

The roots of all seedlings were checked for mycorrhizal infection. Directly after seedling harvesting a length of root was excised and stained for ten minutes using cotton blue in lactic acid. Presence or absence of intracellular coils was determined using a high power microscope.

3.2.4 Determination of total plant N and P concentration

Between 0.9 – 3.0 mg of dried shoot was digested in 0.5 ml 1:1.2 (w/v) H_2O_2 (100 volumes)/H_2SO_4 mixture at 375°C for at least one hour, or until completely digested, following Allen (1989). The resulting digests were diluted to 20 ml with deionised water. Blank digests were also conducted without any

plant material. A bulk blank solution was produced by digesting 2.5 ml of the H_2O_2/ H_2SO_4 solution and diluting to 50 ml using deionised water.

Ammonium-N in the digests was determined using the fluorometric method of Holmes *et al.* (1999). A 0.2 ml sample was diluted to 0.5 ml using blank solution and then either 10 ml borate buffer (40 g $Na_2[B_4O_5(OH)_4]\cdot 8H_2O$ per litre) or 10 ml working reagent (1 l borate buffer + 5 ml 0.8 % (w/v) Na_2SO_3 + 50 ml 4 % (w/v) orthophthaldialdehyde (OPA) in ethanol) was added to two 15 ml test tubes. The resulting mixtures were incubated in the dark for 2.5 hrs, and then the fluorescence was measured using a Wallac 1420 VICTOR multilabel counter (PerkinElmer LAS (UK) Ltd., Beaconsfield, UK). Total nitrogen was determined using a calibration curve over the range 0-4 µg NH_4^+ - N ml^{-1} (Figure 3.3).

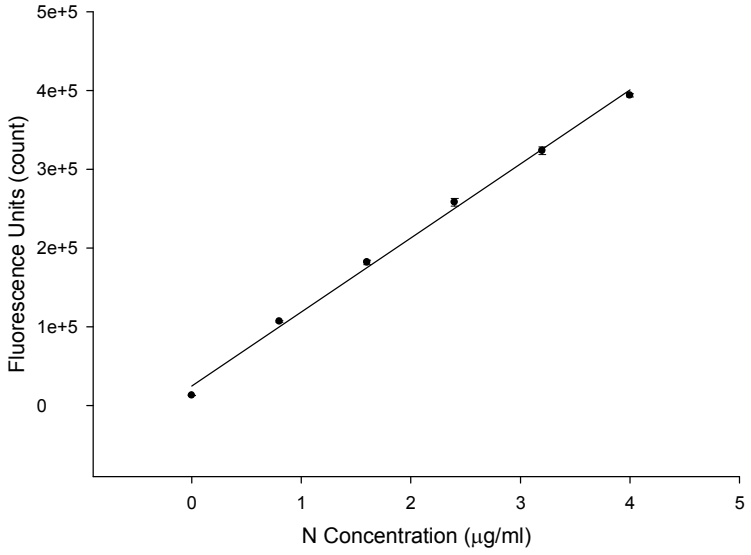


Figure 3.3 Example of linear regression line used to calibrate assay for *C. vulgaris* shoot N concentration. Plotted values are means (*n* = 2) ± 1 SEM.

Phosphorus was determined by the malachite green variant of the methylene blue method as described by Van Veldhoven and Mannaerts (1987). An aliquot of 1.2 ml of the diluted acid digest was diluted further in the ratio 1:1.5

using blank digest solution and then 0.6 ml of Van Veldhoven and Mannaert's (1987) reagent A (1.75 % (w/v) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 6.3N H_2SO_4) was added. After ten minutes 0.6 ml of Van Veldhoven & Mannaerts (1987) reagent C (0.035% (w/v) malachite green in 0.35% (w/v) PVA) was added. After a further 30 min the absorbance was measured at 610 nm using a Pye Unicam SP6-350 visible spectrophotometer. Total phosphorus was determined using a calibration curve over the range 0-0.6 µg PO_4^{3-} - P ml^{-1} (Figure 3.4).

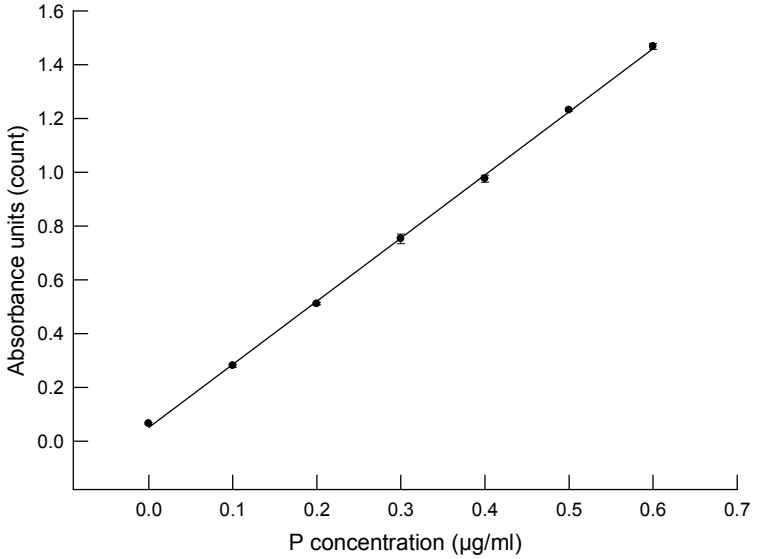


Figure 3.4 Example of linear regression line used to calibrate assay for *C. vulgaris* shoot P concentration. Plotted values are means (*n* = 2) ± 1 SEM.

3.2.5 Statistical Methods

SigmaPlot 11 (Systat Software Inc, California, USA) was used to perform standard statistical analyses and all data were tested for normality and homogeneity of variances and residuals. Relationships between N_W deposition and heathland fragment size and *C. vulgaris* S_W, $[N]_{shoot}$ and $[P]_{shoot}$ and $([N]:[P])_{shoot}$ were investigated using linear regression. N_W was selected in

preference of N_D because modelled N_W data are based on measured deposition values, whereas N_D is derived from transfer models and hence modelled interpolated values contain large uncertainties (Magnani *et al*, 2007). Where test assumptions were not met, data were log_{10} - transformed. Relationships between *C. vulgaris* S_W, $[N]_{shoot}$ and $[P]_{shoot}$ and $([N]:[P])_{shoot}$ were tested against N_T, N_W, N_D, NH_3 concentration, rainfall, latitude, longitude and heathland patch size using multivariate generalized linear models (GLMs). GLMs used transformed or normalised data where appropriate and a minimum adequate model was derived using likelihood ratio deletion tests. Total N deposition incorporates N_W and N_D deposition, so these variables were included in a separate model for comparison. All GLM models were built in R v.2.11.0 (R Foundation for Statistical Computing, Vienna, Austria, 2011).

3.3 Results

The mean initial mass of *C. vulgaris* seedlings upon transfer to the pots was 0.05 ± 0.02 mg. Of the 514 seedlings transplanted, 458 survived for the duration of the experiment (89%). The dry-mass of the individuals differed markedly within pots, possibly due to genetic variation amongst the *C. vulgaris* individuals. S_W, $[N]_{shoot}$ and $[P]_{shoot}$ varied substantially between sites along the N_W deposition gradient (Figure 3.5 and 3.6). The roots of all individuals were infected with mycorrhizal endophyte. Table 3.1 summarises the minimum adequate model for the test variables. All relationships were in a positive direction. N_T and N_D generally explained more variation across all test variables than N_W. There were no significant relationships between $([N]:[P])_{shoot}$ and of any of the variables tested (Table 3.1, Figure 3.6c).

3.3.1 Relationships with N deposition

Analysis of the GLMs indicated that N_D, N_T, and to a lesser extent, N_W were significantly related to *C. vulgaris* S_W, $[N]_{shoot}$ and $[P]_{shoot}$ (Table 3.1). The GLM estimated that S_W increased by 1.75 mg, and $[P]_{shoot}$ increased by 0.005% with each kg of N_D deposition. $[N]_{shoot}$ was estimated to increase by 0.12% with each kg N_W deposition. There was no significant effect of N enrichment on $([N]:[P])_{shoot}$ across the N deposition range studied. S_W was positively related to N_W (Figure 3.5; r^2 = 0.229, P = 0.013, DF = 25). Figure 3.5 illustrates substantial residual variation with modelled N_W deposition accounting for only 23 % of the variation in S_W.

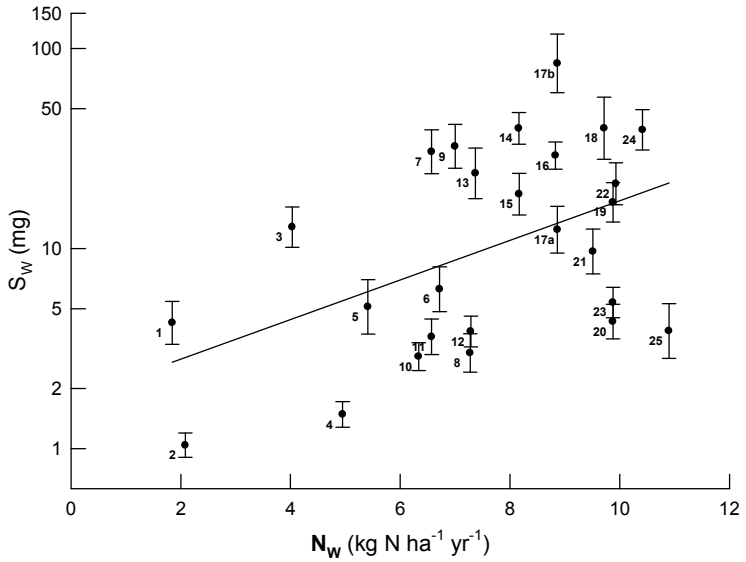


Figure 3.5 Relationship between shoot dry-mass of *C. vulgaris* seedlings and annual mean wet N deposition (N_w) at the 25 study sites. Plotted values are means for each site (n = 10) ± 1 SEM.

Table 3.1 Summary of minimum adequate GLMs for *Calluna vulgaris* variables. Explanatory variables used in Model 1: Total N deposition (N_T), NH_3 concentrations, rainfall, latitude and longitude (interaction and as main effects), and patch size (log_{10} transformed). Explanatory variables used in Model 2: Total wet (N_W) and total dry (N_D) N deposition, NH_3 concentrations, rainfall, latitude and longitude (interaction and as main effects), and patch size (log_{10} transformed). For all variables *DF* = 25.

	Model 1: N_T and NH_3						Model 2: N_W, N_D, and NH_3					
		Parameter Estimates		Model Building Results				Parameter Estimates		Model Building Results		
Variable	**Optimal Model**	**Estimate (± 1 SE)**	***P* Value**	**AIC**	***P* Value**	r^2	**Optimal Model**	**Estimate (± 1 SE)**	***P* Value**	**AIC**	***P* Value**	r^2
S_W				227.11	0.045	0.267				224.54	0.01	0.241
	N_T	1.20 (0.57)	0.045				**N_D**	1.75 (0.85)	0.01			
$[N]_{shoot}$	-	-	-	-	-	-				38.94	0.01	0.292
							N_W	0.12 (0.39)	0.01			
$[P]_{shoot}$	-	-	-	-	-	-				-83.11	0.03	0.108
							N_D	0.005 (0.002)	0.03			
$([N]:[P])_{shoot}$	-	-	-	-	-	-	-	-	-	-	-	-

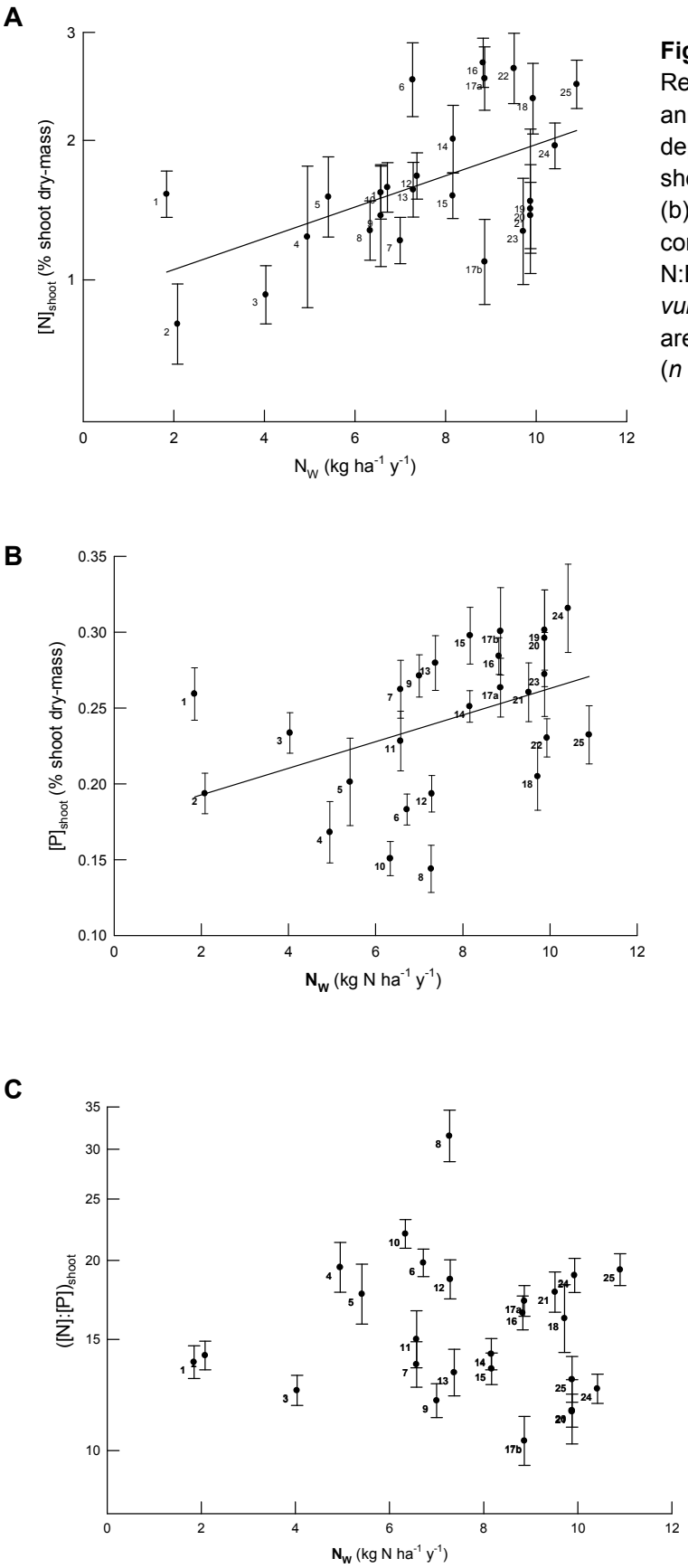


Figure 3.6
Relationships between annual mean wet N deposition (N_W) and (a) shoot N concentration, (b) shoot P concentration, and (c) N:P mass ratio in *C. vulgaris*. Plotted values are means for each site (n = 10) ± 1 SEM.

$[N]_{shoot}$ and $[P]_{shoot}$ at each site were significantly positively related to N_W (Figures 3.6a; r^2 = 0.292, P = 0.004, DF = 25, and 3.6b; r^2 = 0.188, P = 0.027, DF = 25). There was no relationship between $([N]:[P])_{shoot}$ and N_w (Figure 3.6c; r^2 = <0.01, P = 0.805, DF = 25).

3.3.2 Relationship with heathland patch size

Heathland patch size was not related to any form of N deposition or *C. vulgaris* tissue chemistry variable. There was, however, a significant negative response of S_W to increased patch size (Figure 3.7; r^2 = 0.169, P = 0.037, DF = 25).

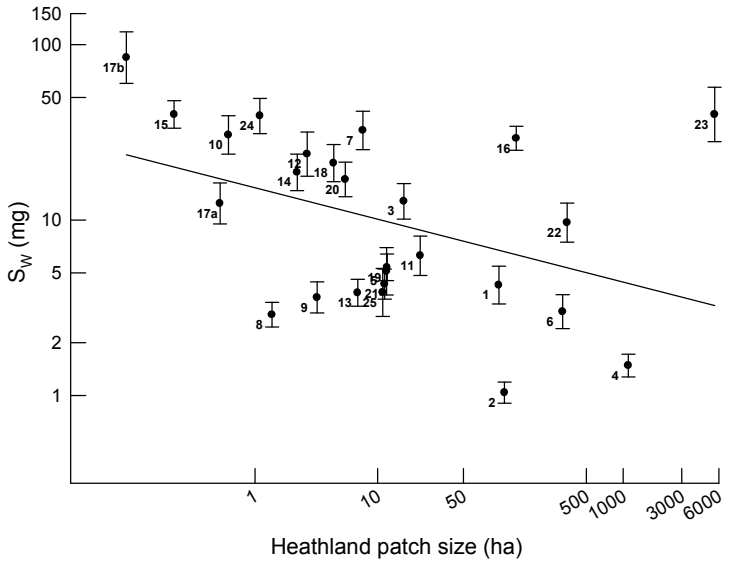


Figure 3.7 Relationship between shoot dry-mass of *C. vulgaris* seedlings and heathland patch size at the 25 study sites. Plotted values are means for each site (n = 10) ± 1 SEM.

3.3.3 Examination for mycorrhizal infection

All roots were infected with mycorrhizal fungi. Notwithstanding the small size of root samples available, there were no obvious differences between sites in the level of infection. Upon observation of a sub-sample of root fragments

across the study sites there appeared to be no influence of N_W deposition on the level of mycorrhizal colonisation at the heathlands studied (Figure 3.8).

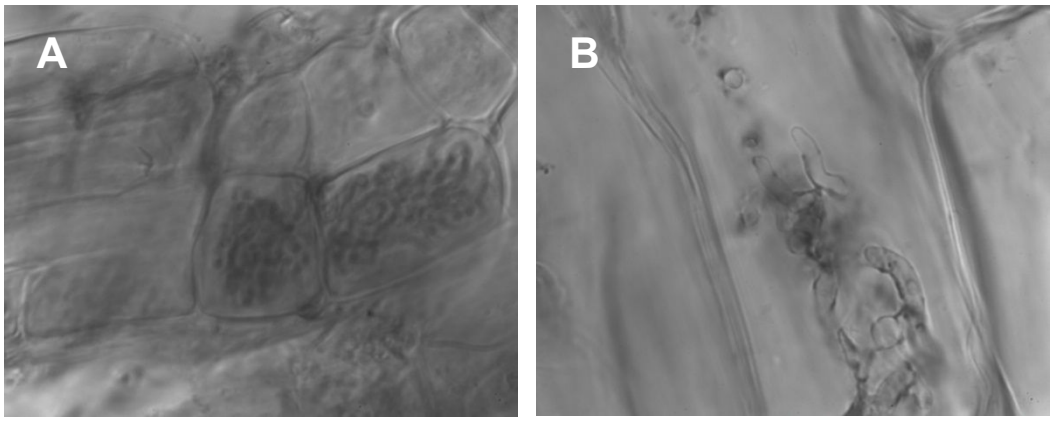


Figure 3.8 Cortical cells of *Calluna vulgaris* hair roots stained with lactophenol cotton blue revealing the presence of intra-cellular coils typical of ericoid mycorrhizas at (A) 600 x magnification, and (B) 1000 x magnification.

3.4 Discussion

Despite the small N_W deposition range examined in this study (1.86 - 10.90 kg N ha^{-1} y^{-1}), relative to the national range (1.86 - 40.40 kg N ha^{-1} y^{-1}), significant relationships between N deposition and *C. vulgaris* growth and shoot chemistry were found in the bioassay. Relationships were in a positive direction, which suggests that N enrichment increased growth and shoot nutrient concentrations in this key heathland species. In view of the extensive evidence of such effects from controlled fertiliser experiments (eg. Aerts, 1989; Pitcairn *et al*., 1995; Power *et al*., 1995; Uren *et al*., 1997) it seems reasonable to assume that these relationships are causal.

The range of $[N]_{shoot}$ (2.74 - 4.70% *C. vulgaris* dry-mass) can be compared with those found in Pitcairn *et al.'s* study (1995) which ranged between 0.98

and 2.66 % shoot dry-mass at sites subjected to total N deposition of 10 - 33 kg N ha^{-1} y^{-1}. Shoot N concentrations were, however, higher than those found by Kirkham (2001), which ranged from approximately 1.2 to 1.5 % dry-mass in *C. vulgaris* shoots subjected to total N deposition of 18.6 to 33.8 kg N ha^{-1} y^{-1}, and Pilkington *et al*. (2005c), who found similar values in moorland vegetation subjected to artificial additions of N upto 120 kg ha^{-1} y^{-1}. Tissue N concentration in *C. vulgaris* is highest in young plants (Robertson & Davies, 1965), and varies seasonally, with the highest concentrations found during summer (Brunsting & Heil, 1985; Thomas, 1937). In the present study *C. vulgaris* was grown under a daytime temperature of 18°C, and was harvested after 18 weeks of growth. N concentrations in *C. vulgaris* were measured throughout the year for all growth stages by Kirkham (2001), and in March for all growth stages by Pilkington *et al.* (2005c). This may explain the higher N concentration values seen in the present study.

$[P]_{shoot}$ (0.14 - 0.32% *C. vulgaris* shoot dry-mass) were similarly higher than those documented in the literature, possibly due to the younger plants in the bioassay having less woody tissue than samples taken from the field. Pilkington *et al*. (2005c) found that P concentrations in *C. vulgaris* were *c.* 0.1% of dry-mass. Similar values were demonstrated by von Oheimb *et al*. (2010) in a heathland subjected to additions of 50 kg N ha^{-1} y^{-1}. $([N]:[P])_{shoot}$ recorded in the present study was in the range reported by Kirkham (2001), von Oheimb *et al*. (2010) and Pilkington *et al*. (2005c).

The relationships between N deposition and *C. vulgaris* S_W, $[N]_{shoot}$ and $[P]_{shoot}$ reflect those found by Jones & Power (2011) in 32 lowland heathland sites in Britain with N_T values in the range 13.3 - 30.8 kg N ha^{-1} y^{-1}. A majority of relationships in the current study were stronger than those found in Jones & Power (2011) possibly due to the use of *C. vulgaris* to assay soil in a

controlled environment in the current study, rather than the collection of shoot samples from the field. Aerts (1989) demonstrated that *C. vulgaris* grows faster under N enrichment, and this has been confirmed here. Pitcairn *et al*. (1995) reported a positive relationship between N deposition and plant tissue N concentration, and Rowe *et al.* (2008) reported a significant positive relationship between N deposition and P concentrations in *C. vulgaris* shoots. This may have been due to increased N leading to greater root/soil phosphatase activity or mycorrhizal colonisation.

This study has confirmed that, across the 25 lowland low-rainfall heathland sites investigated, N enrichment has led to increased availability of N, and increased uptake of P by *C. vulgaris.* The GLM indicated that $[N]_{shoot}$ was estimated to increase by 0.12% with each kg N_W deposited, and $[P]_{shoot}$ increased by 0.005% with each kg N_D deposited. N enrichment has frequently been shown to result in the stimulation of phosphatase activity in soil/root systems, mosses and lichens (Hogan *et al*., 2010a; Arróniz - Crespo *et al*., 2008) and this has been interpreted as evidence of a shift from N limitation to P limitation (Pilkington *et al.,* 2005b; Rowe *et al*., 2008). The lack of a relationship between N deposition and shoot $([N]:[P])_{shoot}$ in this bioassay indicates, however, that N_W has not led to a shift from N to P limitation at the sites studied. Jones and Power (2011) similarly found no relationship between N deposition in any form and *C. vulgaris* shoot N : P ratio over a similar N deposition range to that in the present study. Pilkington *et al*. (2005c) found a positive relationship between N : P ratio and total N deposition, but this was in response to ecologically unlikely N deposition rates up to 120 kg N ha^{-1} y^{-1}. This indicates that a shift to P limitation might not yet have occurred at the sites studied here, but perhaps could in the future with continuing N enrichment and/or if N deposition rates substantially increased. At present,

however, it appears that the current soil reserves of P remain sufficient within the N deposition range studied.

Linear regressions showed that variation in *C. vulgaris* S_W and shoot chemistry could not be completely explained by N deposition in any form; there remained unexplained variance. The GLMs indicated that *C. vulgaris* dry-mass and shoot chemistry variables were not significantly linked to annual rainfall or site latitudinal or longitudinal location, and these factors could not explain any more of the variation. Management intervention could be substantially affecting soil fertility. Due to a lack of reliable records and the temporal and spatial variability in the application of various management techniques, relationships between intervention and soil fertility proved impossible to quantify.

There was, however, a significant response of *C. vulgaris* shoot dry-mass to heathland patch size. Piessens *et al*. (2006) demonstrated that for heathlands surrounded by cropland or forest nutrient concentrations were higher in soils in marginal zones of up to 8 m from the perimeter. At the patches surrounded by forest, *C. vulgaris* was much less prominent near the edge of the patch, and almost absent in sites surrounded by cropland. The enrichment of heathland soils at the edge of patches by ingress of nutrients from the surrounding matrix, through ground water, leaf litter or aerosol/dust deposition, may be reducing patch sizes by conversion of heathland to grassland or woodland communities. Power *et al*. (1998) showed that nitrogen enrichment increases the sensitivity of *C. vulgaris* to frost, drought and *Lochmaea suturalis* (heather beetle) damage. Alonso & Hartley (1998) suggest that *C. vulgaris* is only likely to be outcompeted by graminoids if there are gaps in the canopy which result from damage or death of *C. vulgaris* individuals. Thus, the increased sensitivity of ericoids at heathland edges due to soil N and P

enrichment may facilitate the change from heathland to grassland plant communities, consequently reducing fragment area. The negative relationship between *C. vulgaris* growth and heathland fragment area found in the present study suggests that soil fertility is higher in smaller heathland fragments. Soils were taken from central areas, however, not margins. This might have increased the contrast between larger and smaller patches as the central areas of smaller patches may receive greater nutrient inputs from the surrounding matrix than larger patches. This warrants further investigation.

4

The Relationship Between Soil Phosphomonoesterase Activity and Modelled Nitrogen Deposition

4.1 Introduction

Chapter 3 reported significant positive relationships between modelled N deposition and *Calluna vulgaris* growth and uptake of N and P across 25 lowland heathland sites. There was, however, no relationship between N_W deposition and $([N]:[P])_{shoot}$. Previous research suggests that N enrichment can induce P limitation if increased demand for P is not satisfied by soil reserves or atmospheric P input (Gress *et al*., 2007). Kirkham (2001), for example, demonstrated a significant positive relationship between N deposition in the range 18.6 to 33.2 kg N ha^{-1} y^{-1} and shoot N:P ratios in six moorland species in Britain, and proposed that this is indicative of a shift from N to P limitation in response to N enrichment. Güsewell (2004) suggests that in plants an N:P ratio <10 indicates N limitation, and >20 corresponds to P limitation. A majority of the values found in the present study fall within this range, suggesting that neither N nor P limitation is prevalent across the N_W range studied. This finding was supported by Jones & Power (2011), who demonstrated the same lack of a relationship between N deposition in the range 13.3 to 30.8 kg N ha^{-1} y^{-1} and N:P ratio in *C. vulgaris*. Another indicator of P limitation is an increase in the activity of PME in soil root systems. In this chapter the relationship between PME activity in heathland soils and N_W is investigated to further understand the effects of pollution on British heathlands.

Plants are dependent on mineralised phosphate as their source of P (Duff *et al*., 1994), and this is acquired directly from soil through plant roots. Phosphorus uptake can be enhanced in response to low mineralised phosphate availability through various mechanisms including the formation of root hairs or cluster roots (Lamont, 2003; Shane, *et al*., 2003), or via increased mycorrhizal infection (Bolan, 1991; Clark & Zeto, 2000). In addition, organic P can be made available to plants by the activity of surface bound and secreted phosphatases produced by mycorrhizae and soil micro-organisms (Jansson *et al*., 1988; Kritzler & Johnson, 2010). This family of enzymes, which includes phosphomonoesterases, phosphodiesterases and phosphotriesterases, catalyse the hydrolysis of organic phosphorus compounds (Jansson *et al*., 1988).

Previous field and laboratory studies have demonstrated that PME activity increases in response to P limitation (Treseder & Vitousek, 2001). Johnson *et al*. (1998) found that soil PME activity increased with additions of 40, 80 and 120 kg N ha^{-1} y^{-1} as NH_4NO_3 on a *Calluna vulgaris* - *Vaccinium myrtillus* heathland. Phoenix *et al*. (2003) also demonstrated that additions of 35 and 140 kg N ha^{-1} y^{-1} caused a two-fold increase in root-surface PME activity on a calcareous grassland. Hogan *et al*. (2010) and Arróniz-Crespo *et al*. (2008) discovered increases in PME activity in cryptogams under N enrichment. Jones & Power (2011), however, found no relationship between soil PME activity and N deposition at a range of heathlands in Britain with N deposition rates between 13.3 and 30.8 kg N ha^{-1} y^{-1}, although there was a weak positive response in litter PME activity. Johnson *et al*. (1999) suggested that ammonium enrichment is more important than nitrate enrichment for stimulating root-surface PME activity. Conversely, Phoenix *et al*. (2003) found that nitrate enrichment of soil stimulated PME activity, but ammonium

enrichment did not. Studies indicate that P enrichment retards PME activity (e.g. Hogan *et al*., 2010; Johnson *et al*., 1999; Phoenix *et al*., 2003), possibly due to suppression of enzyme production and competitive inhibition of the active site by orthophosphate (Hunter & McManus, 1999; Kroehler & Linkins, 1988). These studies indicate that PME activity can be stimulated by N enrichment within the deposition range found in mainland Britain.

A change in N:P ratio toward P limitation has been shown to drive plant species composition changes in heathlands with greater dominance of graminoids which are better adapted to low P availability (Fujita *et al*., 2010; Kirkham, 2001). Roem & Berendse (2000) found that *Molinia caerulea* was able to competitively exclude ericoids to a greater extent in P limited heathlands when compared with sites with no P limitation. It is therefore important to understand whether enhanced PME activity could explain the relationship between $([N]:[P])_{shoot}$ and N deposition in Chapter 3 that suggested no N induced P limitation at the heathland sites studied. This chapter reports investigations into the relationship between heathland soil PME activity and modelled N_W within the range 1.85 - 10.90 kg N ha^{-1} y^{-1} at 25 low-rainfall lowland heathland sites in Britain. Relationships between soil PME activity and S_W, $[N]_{shoot}$ and $[P]_{shoot}$ and $([N]:[P])_{shoot}$ are also examined. Assay conditions were optimised for pH and substrate concentration.

4.2 Materials and Methods

4.2.1 Soil collection and pre-treatment

Ten replicate soil samples were collected from each heathland site (Table 2.1) between 22 November 2010 and 07 April 2011. Sampling locations were selected using the same method described in Chapter 2. Soil was taken to a depth of 1 cm from a 10 x 10 cm quadrat and passed through a 3 mm sieve in the field and waste debris returned to the excavation hole. The soil samples were then returned to the laboratory and stored at *c.* 5°C for a maximum of three days.

4.2.2 Determination of phosphomonoesterase activity

PME activity was determined using *p*-nitrophenyl phosphate (*p*NPP) as substrate, as described by Tabatabai and Bremner (1969). A sample of 0.25 g field-moist soil was added to 4 ml deionised water, and the assay initiated by adding 1 ml *p*NPP substrate solution. A 50 mM *pNPP* solution was used in preliminary assays and a 40 mM *p*NPP solution was used in the final survey, yielding concentrations in the assay media of 10 mM and 8mM *p*NPP respectively (see Chapter 4.3). Samples were then placed in a shaking water bath at 15°C for 30 minutes in the dark. The reaction was terminated by the addition of 4 ml 1 M NaOH and 1 ml 0.5 M $CaCl_2$. A 1.5 ml sample of the supernatant was centrifuged in a 2 ml Eppendorf tube at 3000 rpm for five minutes. The absorbance of the supernatant was then measured at 410 nm using a NanoDrop ND-1000 spectrophotometer (Labtech International Ltd, Ringmer, East Sussex). Solutions of *p*-nitrophenol were used to calibrate the assay (Figure 4.1).

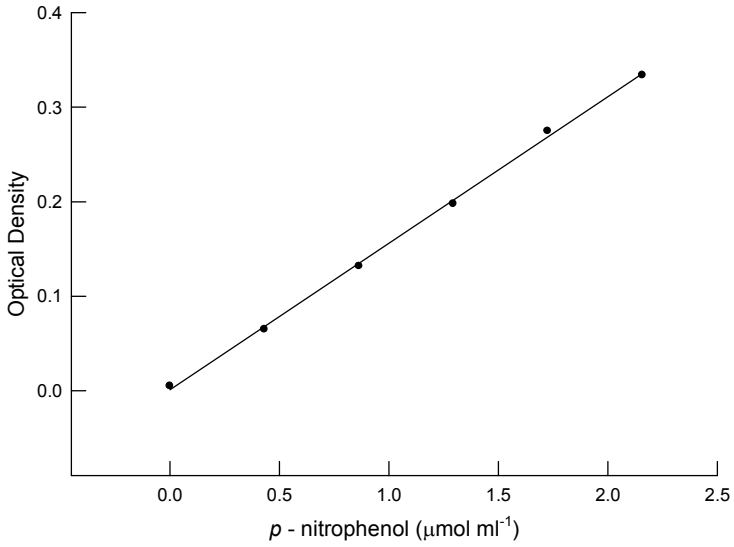


Figure 4.1 Relationship between the concentration of nitrophenol in the assay solution and optical density following colour development.

Control assays were used to correct for interference due to absorption by *p*NPP and any non-enzymatic substrate hydrolysis by following the above method, but adding the *p*NPP after the terminator solutions. Enzyme activity was expressed as either µg substrate hydrolysed g^{-1} dry-mass soil h^{-1} or µg substrate hydrolysed mg^{-1} microbial biomass carbon h^{-1} (see below). Dry mass of soil samples was estimated using wet mass/dry mass ratios determined for all soil samples following oven drying of 1 g sub-samples at 80°C for 24 hours.

4.2.3 Determination of microbial biomass carbon

Microbial biomass carbon was quantified using a modified chloroform fumigation extraction method after Jenkinson & Powlson (1976) and Vance *et* al. (1987). Samples of 10 g field-moist soil were fumigated for 24 h in the dark in a chloroform atmosphere within a pre-evacuated desiccator. Microbial biomass carbon was extracted by adding 50 ml 0.5 M K_2SO_4 to the fumigated soil and shaking for 30 min. The solution was then filtered through Whatman

No. 42 filter paper. The supernatant was stored at -18°C until analysis. Non-fumigated control soils were extracted using the same method. Total organic carbon (TOC) in the extracts were determined using a Shimadzu TOC-V carbon analyser with a TNM-1 module (Shimadzu Corp., Kyoto, Japan).

4.2.4 Optimising assay medium pH

Soils from two randomly selected sites were assayed for PME activity at a range of pH values. The sites selected were Budby Heath and Litcham Common, with N_w values of 8.8 and 10.4 kg ha^{-1} y^{-1} respectively. The pH of the assay medium was varied using citric acid – trisodium citrate buffer as described by Dawson *et al*. (1986) (Table 4.1).

Table. 4.1 pH values of assay media in the range 3.2 – 6.4 prepared using a solution of 0.1 M citric acid (x ml) and 0.1 M trisodium citrate (y ml) per litre (after Dawson *et al*., 1986).

Target pH	Measured pH	*x* ml 0.1 M citric acid	*y* ml 0.1 M trisodium citrate
3.0	3.2	82.0	18.0
3.4	3.6	73.0	27.0
3.8	4.1	63.5	36.5
4.2	4.4	54.0	46.0
4.6	4.8	44.5	55.5
5.0	5.2	35.0	65.0
5.4	5.6	25.5	74.5
5.8	5.9	16.0	84.0
6.2	6.4	8.0	92.0

Soil pH was determined by mixing *c.* 1 g moist soil with *c.* 10 ml deionised water and measured using a Minilab IQ125 pH meter (IQ Scientific Instruments, California, USA).

4.2.5 Optimising substrate concentration

The effect of substrate concentration on PME activity was determined using soils collected from Budby Heath. Assays were conducted with final *p*NPP concentrations in the range 2mM – 10mM.

4.2.6 Statistical Methods

SigmaPlot 11 (Systat Software Inc, California, USA) was used to perform standard statistical analyses. All data were subjected to normality and homogeneity of variance tests and residuals from fitted models were inspected. Non-normal data were $\log_{10}$ transformed or homogenised by applying a fixed variance structure and then analysed using linear regression. Enzyme kinetics were analysed using ligand-binding one-site saturation non-linear regression using the Michaelis–Menten formula. Relationships between PME activity and both *C. vulgaris* and environmental variables were tested using generalized linear models (GLMs). GLMs used untransformed data and a minimum adequate model was derived using likelihood ratio deletion tests. The independent variables included in the initial model were N_W, N_D, NH_3, S_W, $[N]_{shoot}$, $[P]_{shoot}$, $([N]:[P])_{shoot}$, patch size and rainfall, and were tested against PME activity expressed both as soil dry-mass^{-1} h^{-1} and microbial biomass C^{-1} h^{-1}, and soil microbial biomass C. All GLM models were built in R v.2.11.0 (R Foundation for Statistical Computing, Vienna, Austria, 2011).

4.3 Results

4.3.1 Response of PME activity to assay medium pH

PME activity was readily measureable in all soil subsamples from both sites, and at all pH values tested. In both soils enzyme activity generally increased with increasing pH, with maximum values recorded at pH 5.9 and lowest values recorded at pH 3.2 (Figure 4.2). Nonetheless response to pH at Budby was fairly flat and was not significant (r^2< 0.002, P = 0.767, DF = 9). Measured soil pH values at Budby Heath and Litcham Common were 4.9 and 5.1 respectively, and are at, or close to, the measured optimum pH values for PME activity in the soils in question. Soil pH among all 25 sites ranged from

3.5 to 5.6 and was not related to N_W deposition (r^2 = 0.01, P = 0.564, DF = 25). Therefore it was decided to conduct assays in unbuffered assay media to allow the phosphatases to operate at the original soil pH.

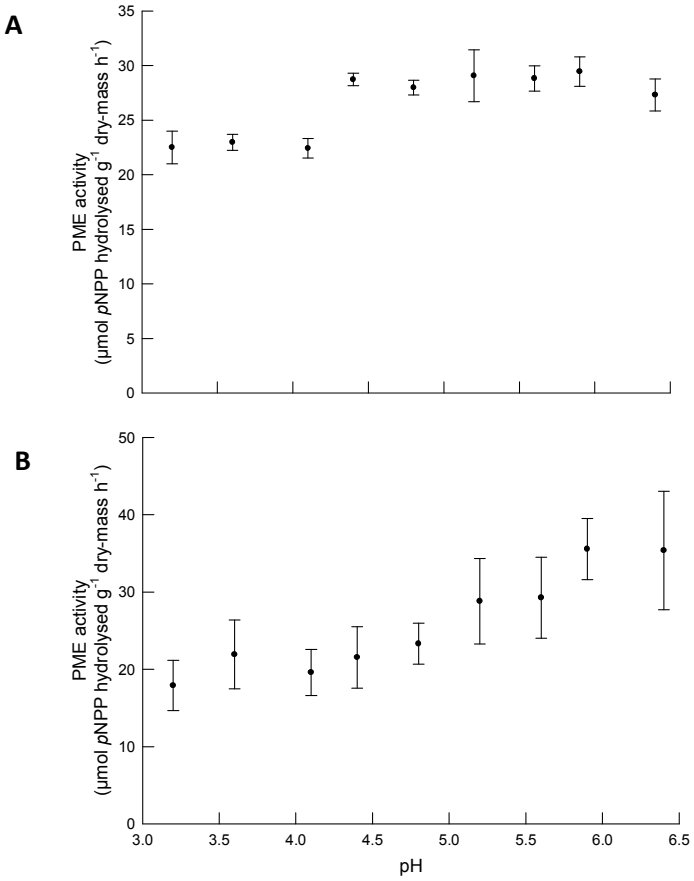


Figure 4.2 The effect of assay medium pH on PME activity in soils collected from (A) Budby Heath and (B) Litcham Common. Assays were performed with 10 mM *p*NPP for 30 min in the dark at 15°C. Plotted values are means (n = 6) ± 1 SEM.

4.3.2 Response of PME activity to substrate concentration

Soil PME activity was readily measurable at all substrate concentrations tested and was positively related to substrate concentration (Figure 4.3; r^2 = 0.914, P <*0.001,* DF = 5). Enzyme activity increased with increasing substrate concentration between 2 – 6 mM *p*NPP and appeared saturated at concentrations >6 mM (Figure 4.3). For all subsequent assays a concentration of 8 mM *p*NPP was used. This concentration was considered likely to saturate

PME activity at most or all sites, but produce a lower blank reading than higher substrate concentrations.

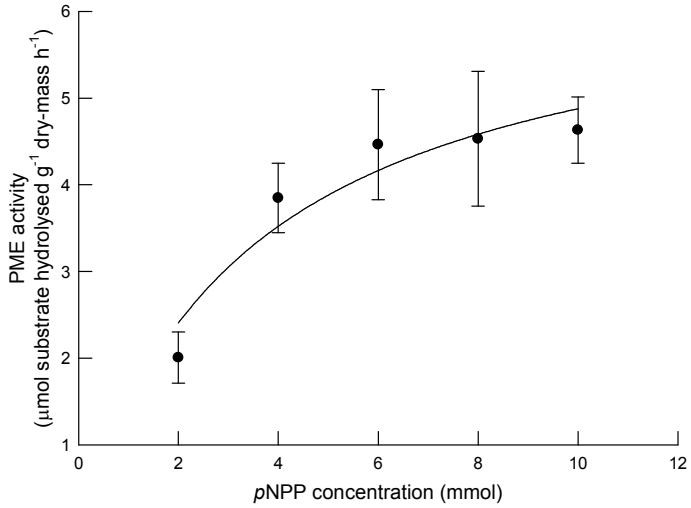


Figure 4.3 Relationship between PME activity and substrate concentration in soil from Budby Heath. Assays were performed at 15°C for 30 minutes in the dark. Plotted values are means (n = 6) ± 1 SEM. The line is a ligand-binding one-site saturation non-linear regression.

4.3.3 Inter-site variation in PME activity

PME activity expressed on a per unit soil dry-mass and a per unit soil microbial biomass carbon basis ranged between 0.003 - 0.15 mmol *p*NPP hydrolysed g^{-1} dry-mass soil h^{-1} and 0.13 - 29.44 mmol *p*NPP hydrolysed g^{-1} soil microbial biomass carbon h^{-1}, respectively. PME activities expressed using these different mass bases were strongly related (Figure 4.4; r^2= 0.706, P<0.001, DF = 257).

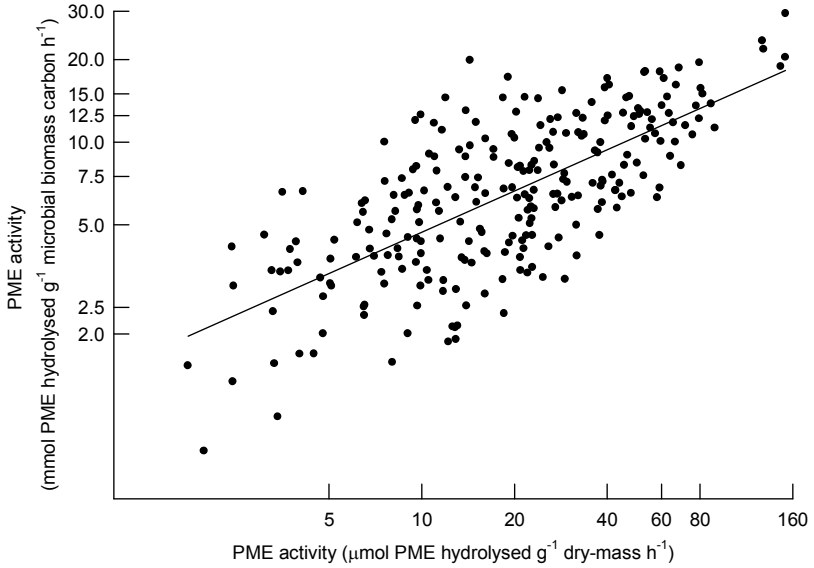


Figure 4.4 The relationship between PME activity expressed as soil dry-mass g^{-1} and microbial biomass carbon g^{-1} (note log scales).PME assays were performed with 8 mM *p*NPP for 30 minutes at 15°C in the dark.

There was no evidence for a confounding effect of date of collection (Figure 4.5; r^2 = 0.004, P = 0.726, DF = 27). The PME activity of soils collected at the beginning and end of the collection period were tested to investigate an effect of seasonal variation in PME activity. Soils from Beacon Hill and Ulverscroft were collected both in November 2010 and April 2011. PME activity on the two collection dates were not significantly different (Figure 4.6; t = 0.462, P = 0.649, DF = 18 and U = 48, P = 0.910, DF = 18 respectively).

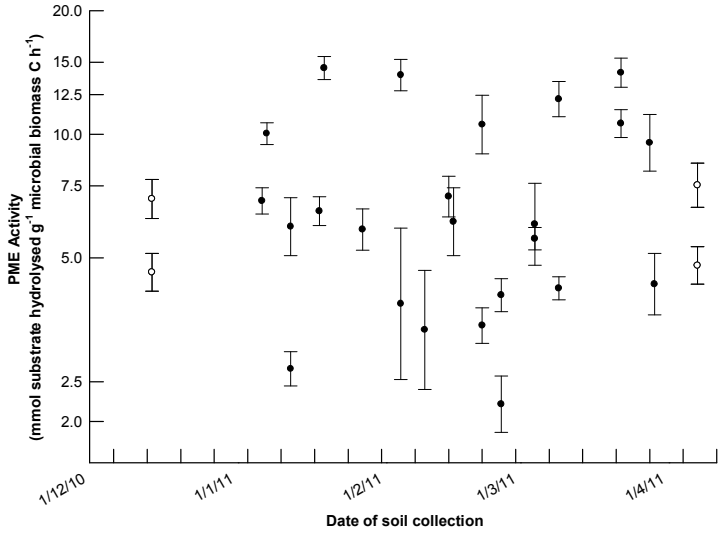


Figure 4.5 The relationship between PME activity and soil collection date. Assays on soils collected from Beacon Hill and Ulverscroft were repeated in December 2010 and April 2011 (open circles). Assays were performed with 8 mM *p*NPP for 30 minutes at 15°C in the dark.

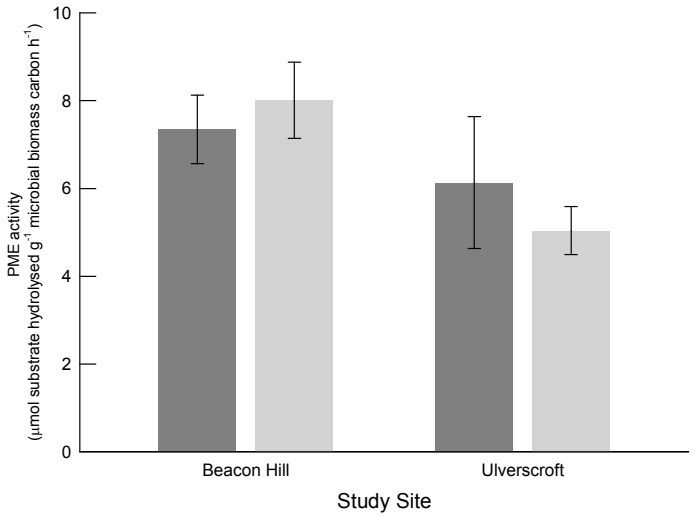


Figure 4.6 The relationship between soil collection date and PME activity in soils from Beacon Hill I and Ulverscroft collected on 14/12/2010 (dark bars) and on 07/04/12 (light bars). Assays were performed with 8mM *p*NPP for 30 minutes at 15°C in the dark.

Inter-site variation in PME activity expressed on a per unit soil microbial biomass C basis was unrelated to N_W deposition (Figure 4.7a; r = -0.213, P = 0.296, DF = 25), and all of the measured outcomes of the soil bioassay (Chapter 3): S_W (Figure 4.7b; r = -0.211, P = 0.300, DF = 25), $[N]_{shoot}$ (Figure 4.7c; r = 0.058, P = 0.778, DF = 25), $[P]_{shoot}$ (Figure 4.8d; r = -0.345, P = 0.08, DF = 25), and $([N]:[P])_{shoot}$ (Figure 4.7e; r = 0.346, P = 0.09, DF = 25). There was no significant effect of nitrogen deposition form (Table 4.2). The GLM analysis revealed no relationships between PME activity expressed on a per unit soil microbial biomass basis, and any of the variables tested (Table 4.3).

When PME activity was expressed on a per unit soil dry-mass basis, however, it was found to be significantly related to several variables: N_W deposition (Figure 4.8a; r = -0.454, P = 0.02, DF = 25), N_D deposition (r = -0.487, P = 0.01, DF = 25), NH_3 concentration (r = 0.407, P = 0.04, DF = 25), $[P]_{shoot}$ (Figure 4.8d; r = -0.680, P = <0.01, DF = 25), and $([N]:[P])_{shoot}$ (Figure 4.8e; r = 0.546, P = <0.01, DF = 25) were all significantly related to PME activity expressed on a per unit dry-mass basis (Table 4.2). It should be noted, however, that it is possible that some of these relationships have been detected by chance due to the large number of correlations tested. The GLM analysis suggested that $[P]_{shoot}$ was the only variable which could explain variation in PME activity on a per unit dry-mass basis (Table 4.3).

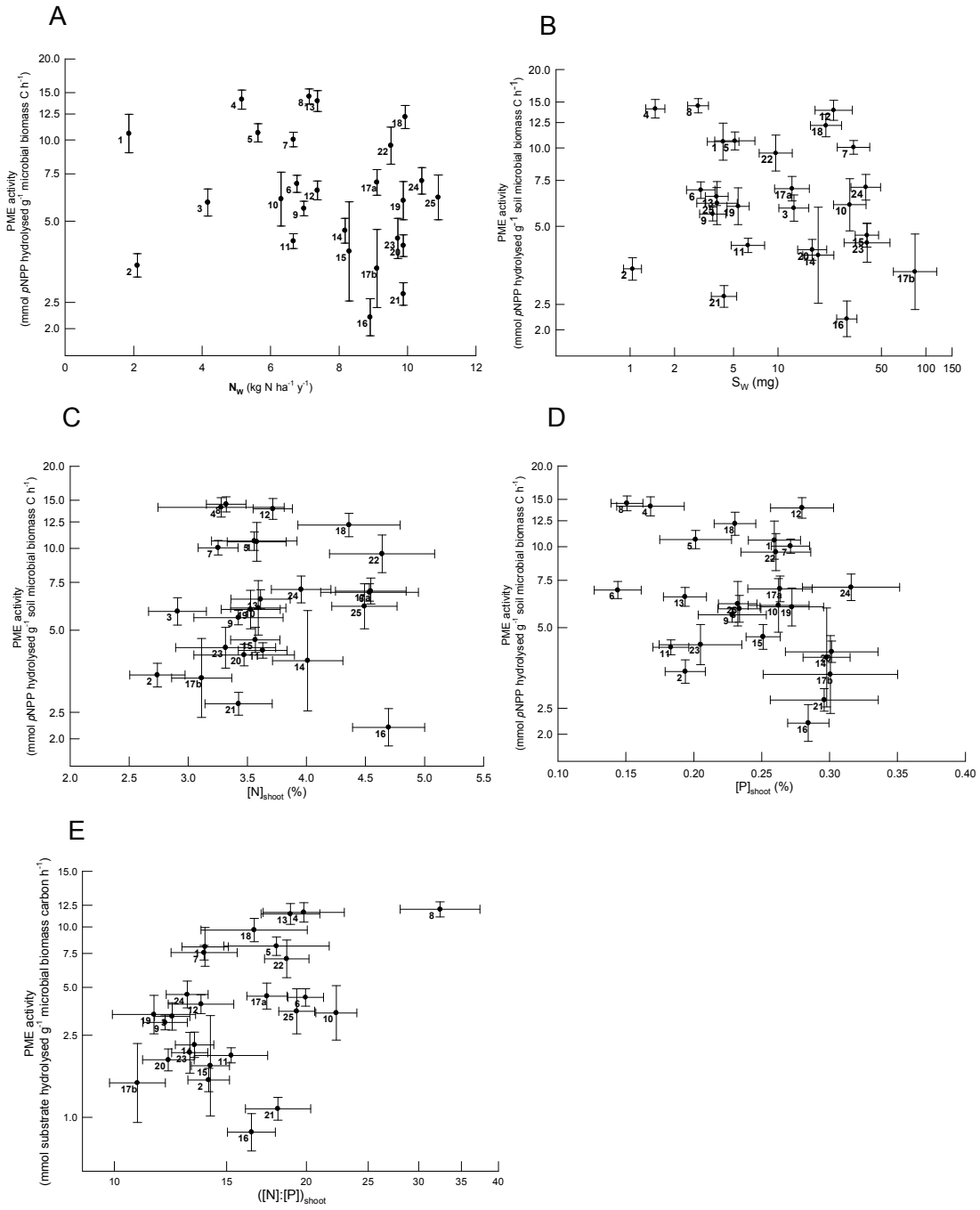


Figure 4.7 The relationship between soil PME activity on a per unit microbial biomass basis (note log scales) and (A) wet deposited N (N_W), (B) *C. vulgaris* shoot dry-mass, (C) N concentration, (D) P concentration, and (E) N:P mass ratio. Assays were performed with 8 mM *p*NPP for 30 minutes at 15 °C in the dark. Plotted values are means (n = 10) ± 1 SEM. Symbol numbers represent the site numbers reported in Chapter 2 (Table 2.1).

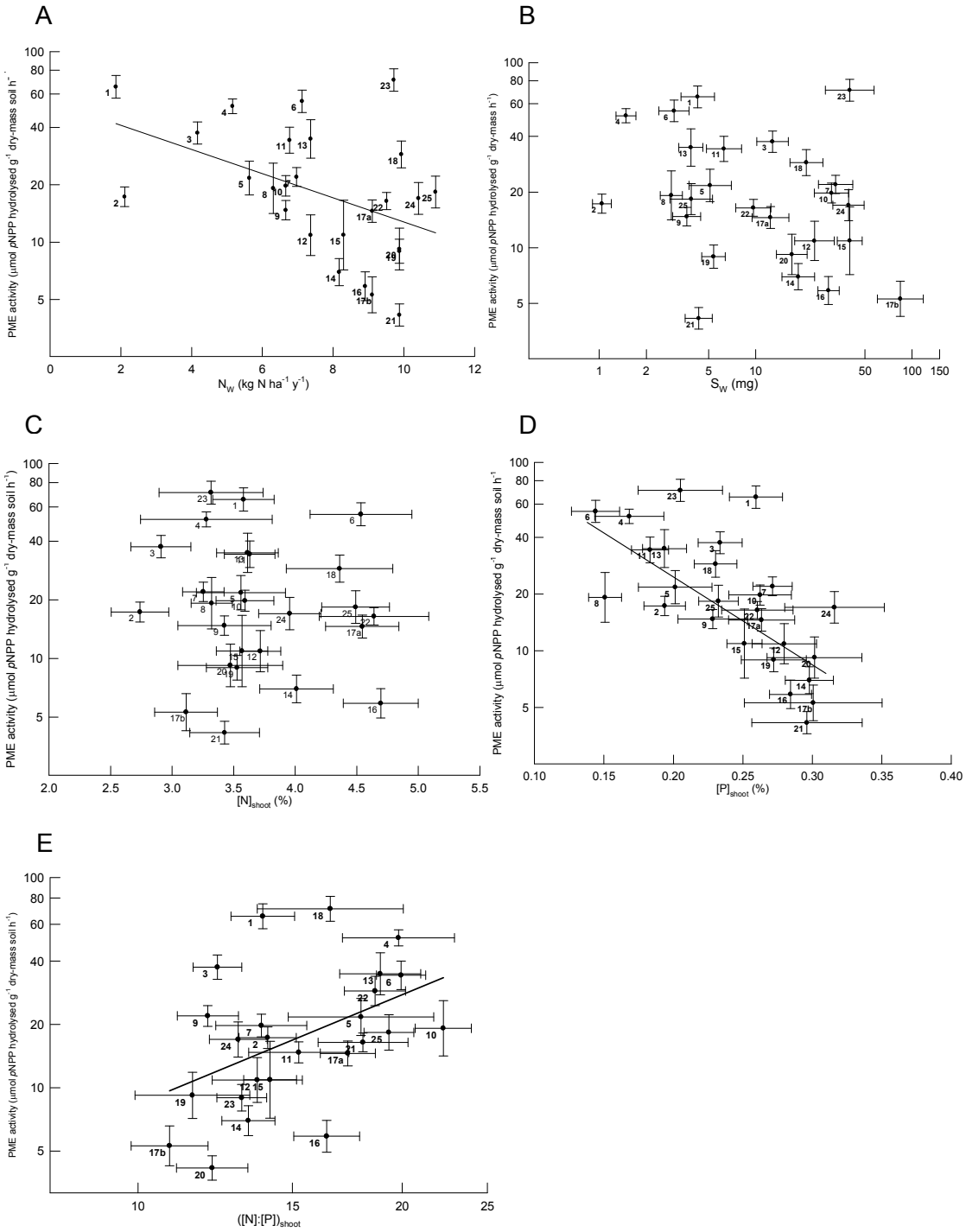


Figure 4.8 The relationship between soil PME activity on a per unit soil dry-mass basis (note log scale) and (A) wet deposited N (N_W), (B) *C. vulgaris* shoot dry-mass, (C) N concentration, (D) P concentration, and (E) N:P mass ratio. Assays were performed with 8 mM *p*NPP for 30 minutes at 15 °C in the dark. Plotted values are means (n = 10) ± 1 SEM. Symbol numbers represent the site numbers reported in Chapter 2 (Table 2.1), and the lines represent simple linear regression models.

Table 4.2 Pearson Moment correlation values (*r*) between PME activity expressed both on a per unit soil dry-mass h^{-1} (PME DM), and a per unit microbial biomass C h^{-1} (PME MBC) basis, and wet inorganic N deposition (N_W), dry inorganic N deposition (N_D), modelled NH_3 concentration, *Calluna vulgaris* shoot dry-mass (mg), shoot N and P concentrations (% shoot dry-mass), N:P mass ratio in shoot dry-mass, site area (ha) and annual rainfall (mm) across the 25 study sites.

*** - correlation is significant at the *P* ≤0.05 level** **** - correlation is significant at the *P* ≤ 0.01 level**

	N_W	N_D	NH_3	S_W	$[N]_{shoot}$	$[P]_{shoot}$	$([N]:[P])_{shoot}$	Site Area	Rainfall
PME DM	-0.454 *	-0.487 *	0.407 *	-0.309	-0.080	-0.680 **	0.546 **	0.545 **	0.114
PME MBC	-0.213	-0.144	-0.090	-0.211	0.058	-0.345	0.346	0.239	-0.138
MBC	-0.486 *	-0.602 **	-0.529 **	-0.243	-0.076	-0.524 **	0.345 *	0.584 **	0.306

Table 4.3 Summary of optimal GLMs for phosphomonoesterase expressed on a per unit dry-mass h^{-1} and a per unit microbial biomass C h^{-1} basis and soil microbial biomass C. Independent variables were total wet (N_W) and total dry (N_D) N deposition and NH_3 concentrations, rainfall, latitude and longitude, patch size (log_{10} transformed), *C. vulgaris* dry-mass, shoot N and P concentrations and N:P mass ratio. For all variables *DF* = 25.

		Parameter Estimates		**Model Building Results**		
Variable	**Optimal Model**	**Estimate (± 1 SE)**	***P* value**	**AIC**	***P* value**	r^2
PME DM	-	-	-	5.58	<0.001	0.429
	$[P]_{shoot}$	-27.50 (11.30)	<0.001			
PME MBM	-	-	-	-	-	-
MBC	-	-	-	-12.90	0.002	0.443
	Patch size	1.25 (1.08)	0.007 0.027			
	N_W	-1.47 (1.04)				

4.4 Discussion

The present study found that soil PME activity was not related to N_W deposition when expressed on a per unit microbial biomass C h^{-1} basis, but was moderately negatively related to N_W, N_D deposition and NH_3 concentrations when expressed on a per unit dry-mass h^{-1} basis. The GLM indicated that with each 0.1% decrease in $[P]_{shoot}$ PME activity was estimated to decrease by 27.5 µm *pNPP* hydrolysed g^{-1} dry-mass soil h^{-1}. In line with what was expected there was evidence to suggest that $[P]_{shoot}$ was strongly negatively related to PME. There was no evidence to indicate that PME activity was enhanced in response to N enrichment. This suggests that P reserves in the soil were sufficient to satisfy demand under N enrichment up to 10.90 kg N ha^{-1} y^{-1}.

The rates of PME activity observed in the present study are comparable with those found by Johnson *et al*. (1998) in heathland soil subjected to artificial additions of 4, 8 and 12 kg N ha^{-1} yr^{-1}. In Johnson *et al*'s study mean soil PME activity for all horizons examined ranged between 54 - 118 µmol substrate hydrolysed g^{-1} dry-mass h^{-1} when assayed at 37°C. These values were also comparable to those found by Pilkington *et al.* (2005) who used similar methods. The PME activity rates observed in the present study, however, are substantially lower than those observed by Johnson *et al.* (2010) in mixtures of ombrotrophic peat and *C. vulgaris* litter from sites subjected to artificial N additions of 64 kg N ha^{-1} y^{-1}. Here PME activity ranged between 3.6 x 10^3 and 9.0 x 10^5 µmol substrate hydrolysed g^{-1} dry-mass litter/soil h^{-1} assayed at 37°C, dependent on the N deposition form and on the litter composition. For example, PME activity was highest when litter contained *Sphagnum* species (Johnson *et al*., 2010). In the present work PME activity was in the range 0.34 - 151.71 µmol substrate hydrolysed g^{-1}soil dry-mass h^{-1} when assayed at

15°C. PME rates measured by Jones & Power (2011) were also substantially higher in litter from a range of British heathlands subject to varying atmospheric N deposition loads than those in the present work and ranged from 5 to 45 mmol substrate hydrolysed g^{-1} dry-mass litter h^{-1} when assayed at 37°C. It must be noted that Jones & Power (2011) assayed at a higher temperature than the present study, so greater PME activity would be expected upon comparison.

Soil PME activity expressed on a microbial biomass C basis was not related to nitrogen deposition in any form. At first sight this suggests that soil PME activity is not up-regulated in response to N_W deposition within the range 1.85 - 10.90 kg N ha^{-1} $y^{-1}$and that the higher $[P]_{shoot}$ at the higher N_W values is due to increased P uptake from soil pools. This is consistent with the growth and P content of *C. vulgaris* in the bioassay in which N:P mass ratio was not typical of P limited plants (Chapter 3). A similar study by Jones & Power (2011), also found no response of soil PME activity to total N deposition within the range 13.3 to 30.8 kg N ha^{-1} y^{-1}. Pilkington *et al*. (2005b), however, observed significantly increased activity from 39.6 to 76.68 µmol substrate hydrolysed g^{-1}soil dry-mass h^{-1} in response to long-term artificial additions of ammonium nitrate at rates of 40, 80 and 120 kg N ha^{-1} y^{-1}. Therefore, it appears that the N deposition range in the current investigation did not include N_W values sufficiently high enough to induce P limitation and subsequently stimulate PME activity. In the present study there were significant weak negative relationships between N_W and N_D and PME activity expressed on a per unit dry-mass basis. As this measure takes into account the non-organic component of the soil, such as sand, which does not contribute to soil PME activity, these relationships may be a reflection of the bulk-density of the soil rather than the effect of N deposition on PME activity.

In studies by both Jones & Power (2011) and Pilkington *et al*. (2005b) the values of PME were greater in the litter layer than other parts of the soil horizon, and Pilkington *et al*. (2005b) found that N additions positively affected PME activity in the top 1 cm of the organic soil horizon to a greater extent than the top 2 cm. Johnson *et al*. (1998) observed a two-fold increase in soil PME activity with additions of 40 kg N ha^{-1} y^{-1} in the top 7 cm of soil. In the present study PME activity was measured in the top 1 cm of the organic soil layer, which harbours the highest density of plant roots (Caporn *et al*., 1995; Tinhout & Werger, 1988). These roots, with the associated mycorrhizal fungal symbionts, would be expected to produce the highest rates of extra-cellular phosphatase activity in soil (Leake & Miles, 1996; Straker & Mitchell, 1986).

In a bioassay of heathland soils (Chapter 3) $[N]_{shoot}$ and $[P]_{shoot}$ increased with N_W deposition, but there was no relationship between $([N]:[P])_{shoot}$ and N_W deposition. Here it was shown that there was a significant strongly negative relationship between PME activity expressed on a per unit dry-mass basis and $[P]_{shoot}$, but not on a per unit microbial biomass C basis; although moderately negative the relationship was not significant (P = 0.08). The negative relationship between PME activity and $[P]_{shoot}$ suggests that greater soil PME activity may result from lower soil P availability.

In the present study $([N]:[P])_{shoot}$ was not significantly related to PME activity when expressed on a per unit microbial biomass basis, but there was a strong positive relationship when PME was expressed on a per unit dry-mass basis. It should be noted that Von Oheimb *et al*. (2010) found that the N:P mass ratio of *C. vulgaris* shoots fluctuated annually when regular additions of 50 kg N ha^{-1} y^{-1} and 20 kg P ha^{-1} y^{-1} over five years were applied, possibly due to variations in soil microbial activity during the study (van Meeteren *et al*., 2007). Therefore N:P mass ratio should not be used to predict N or P limitation

unless the values are particularly high, or particularly low. In a review of N:P mass ratios in terrestrial plants, Güsewell (2004) suggests that N:P mass ratios <10 in vascular plants are indicative of N limitation, and values >20 indicate P limitation, but that values in between cannot reliably indicate limitation by either N or P. The N:P ratio values in Chapter 3 suggest that P is not limiting at the heathland sites studied. This is further supported by the lack of a relationship between PME activity expressed on a per unit microbial biomass C basis and N_W deposition seen in the present study. Therefore N induced demand for P must be met by soil inorganic P resources. Previous research has understandably focused on the direct effects of N deposition, such as plant N uptake as investigated in Chapter 3 of the current study. To fully understand the impacts of N enrichment on heathlands, however, the indirect effects on plant nutrition must be investigated. The current research has contributed to the knowledge that PME activity may be related to soil N and P availability. While enhanced PME activity was not seen in heathlands subject to up to 10.90 kg N ha^{-1} y^{-1} it might be evident in heathlands subject to greater N enrichment and this warrants further investigation.

5

Atmospheric Ammonia Concentrations at Eleven Heathland Sites in the East Midlands, UK

5.1 Introduction

Previous chapters have indicated that there are significant positive relationships between N deposition and the growth and tissue chemistry of *C. vulgaris*. There was, however, substantial residual variation in the results. The current study investigates the possibility that NH_3 deposition could account for some of residual variation observed in chapter 3. Ammonia is a major contributor to anthropogenic N emissions into the atmosphere accounting for 55 % of the total N emissions globally in 1990 (Olivier *et al*., 1998). NH_3 emissions in the UK were estimated to total 242 Gg N - NH_3 in 2010, but this is highly uncertain with a ± 20% error (Matejko *et al*., 2009). The primary NH_3 source is farm livestock (40%), with fertilisers (17%), oceans (15%), biomass burning (8%), agricultural crops (7%) and others (13%) accounting for the remainder (Asman *et al*., 1998; Bouwman *et al*., 1997; Erisman *et al*., 2007). Cattle and poultry farming contribute the majority of NH_3 emissions from farm livestock (Misselbrook *et al*., 2000). Misselbrook *et al*. (2000) suggest that excreta from a single cow can emit between 5.61 and 21.84 kg NH_3 y^{-1}, a pig between 3.96 and 4.29 kg NH_3 y^{-1}, a sheep up to 0.60 kg NH_3 y^{-1}, and a hen between 0.19 and 0.37 kg NH_3 y^{-1}. Thus, depending on animal stocking densities, NH_3 emissions from agricultural units can be substantial.

Much of the research into the impact of N deposition on plant communities and soil fertility has been based on modelled N deposition data at a 5 x 5 km

resolution. Sutton *et al*. (2001) illustrated that airborne NH_4^+ aerosol shows little spatial variability, with a smooth interpolated field of atmospheric concentrations decreasing steadily from sources. Irwin & Williams (1988) suggest that, assuming an atmospheric residence time of six days and an average wind velocity of 5 m s^{-1}, NH_4^+ could potentially be deposited up to 2500 km from the emission source. The interpolated NH_4^+ deposition models at a 5 x 5 km resolution used in Chapter 3 and 4 can therefore be assumed to be reliable estimates of actual deposition. The atmospheric residence time of NH_3 gas, on the other hand, can be as little as 2.8 h (Erisman *et al*., 1988), since it is quickly converted to NH_4^+ or dry-deposited close to the emission source (Ferm, 1998). A study by Dragosits *et al*. (2002) of atmospheric NH_3 concentrations within a 5 x 5 km grid square, interpolated to a 50 m grid resolution using a local area dispersion and deposition model, showed that high NH_3 concentrations near a point source, such as a poultry farm, could fall to ambient levels within 2.5 km of the source. Sutton *et al*. (2001) concluded that, in order to accurately model NH_4^+ deposition, only a few tens of measuring stations would be needed, while the high spatial variability of NH_3 deposition would require thousands of monitoring stations in the same area to accurately characterise spatial patterns.

Atmospheric NH_3 enters higher plants almost exclusively through stomata on the leaves, rather than through roots, as in the uptake of wet deposited N (Sutton *et al*., 1992; van Hove *et al*., 1987). NH_3 deposition onto leaves is altered by canopy resistance (Erisman & Wyers, 1993; Sutton *et al*., 1992), and penetration into leaves by cuticular resistance (Jones *et al*., 2007), both of which vary between plant species and with NH_3 concentrations. Two models have been developed to predict NH_3 deposition based on these factors, but there is still significant uncertainty in using these models to estimate NH_3

deposition. For example it is still unknown how canopy and cuticular resistance is affected by atmospheric NH_3 concentrations (Jones *et al*., 2007).

The current critical level for NH_3 concentration, defined as the concentration in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur (Posthumus, 1988) was set at 8 μg m^{-3} in 1993 (Ashmore & Wilson, 1994). Cape *et al*. (2009), however, propose that this should be reduced to 3 ± 1 μg m^{-3} for higher plants. A study by Sheppard *et al*. (2011) demonstrated significant effects of artificial NH_3 applications between the equivalent of 3 and 70 kg N ha^{-1} y^{-1} at an ombrotrophic bog at Whim Moss, Scotland. After three years of exposure, *C. vulgaris* leaves had been bleached, and green cover had been significantly reduced up to 16 m from the NH_3 source. In a field experiment Sheppard *et al*. (2011) showed that, when *C. vulgaris* was subjected to the same deposition values for NH_3 and NH_4^+ in rainfall, visible damage and reduced green cover were far less pronounced under the wet applications. At 60 m from the application source, which was subject to NH_3 concentrations equivalent to 4 μg m^{-3}, *C. vulgaris* showed no visible changes after 7 years of exposure. This atmospheric concentration value is typical of that found in many rural areas in close proximity to agricultural activities (Tang *et al*., 2009). Leith *et al*. (2001) demonstrated that the foliar N content of *C. vulgaris* increased in response to artificial exposure to NH_3 in the range 0 to 90 μg m^{-3} in open-top chambers. Again, the response was more pronounced in relation to NH_3 concentrations compared to the equivalent NH_4^+ additions. This is consistent with the response found by van der Eerden *et al*. (1991) in *C. vulgaris* subject to NH_3 concentrations up to 100 μg m^{-3} in which shoot N concentration increased 4 fold after exposure for 38 weeks.

The extreme spatial variability of atmospheric NH_3 concentrations, and problems with deposition estimates, suggests that there is a need to measure NH_3 concentrations at smaller scales in order to obtain accurate data. Chapter 3 reported significant positive relationships between N_W and the growth and tissue chemistry of *C. vulgaris*. There was, however, substantial residual variation in the results. The current study investigates the possibility that NH_3 deposition could account for some of this residual variation. This chapter, therefore, reports investigations into the relationships between measured NH_3 concentrations ($[NH_3]$) within the range 0.96 to 3.50 µg m^{-3} and the residual variation in S_W, $[N]_{shoot}$ and $[P]_{shoot}$ at 11 low-rainfall lowland heathland sites in the East Midlands region of Britain.

5.2 Materials and Methods

5.2.1 Site selection

Of the 25 heathland sites identified in Chapter 2, 11 sites were used for this study, all of which were located within the East Midlands region of Britain. The sites were selected using the methods described in Chapter 2. As before, all sites were below 300 m elevation and had an annual rainfall within the range 549 - 836 mm y^{-1}.

5.2.2 Sampling apparatus

Atmospheric ammonia was captured using ALPHA (Adapted Low-cost Passive High Absorption) samplers constructed and supplied by CEH Edinburgh (Tang *et al*., 2001). These are passive samplers containing filter paper coated with citric acid, which serves to capture the ammonia, mounted in a circular polyethylene vial, and protected by a 5 µm PTFE membrane over the orifice (Figure 5.1; Tang *et al*. 2001). Two replicate ALPHA samplers were exposed at each site. Samplers were attached to an inverted plant pot saucer

which was fixed to a wooden post at a height of 1.5 m above the ground. Plastic bird-deterrent spikes were mounted on top of the plant saucer to prevent birds from perching and contamination from excreta. The ALPHA samplers were exposed in the field for *c.* 30 days, after which they were replaced by new unexposed units. When not exposed, ALPHA samplers were stored capped in sample bottles at *c.* 5 °C. Ammonia was sampled at each site for a total of 12 months from August 2010 to July 2011, with the exception of Oak Tree Heath, which was sampled for five months from March to July 2011 due to difficulty finding suitable locations to place the post in a highly populated area.

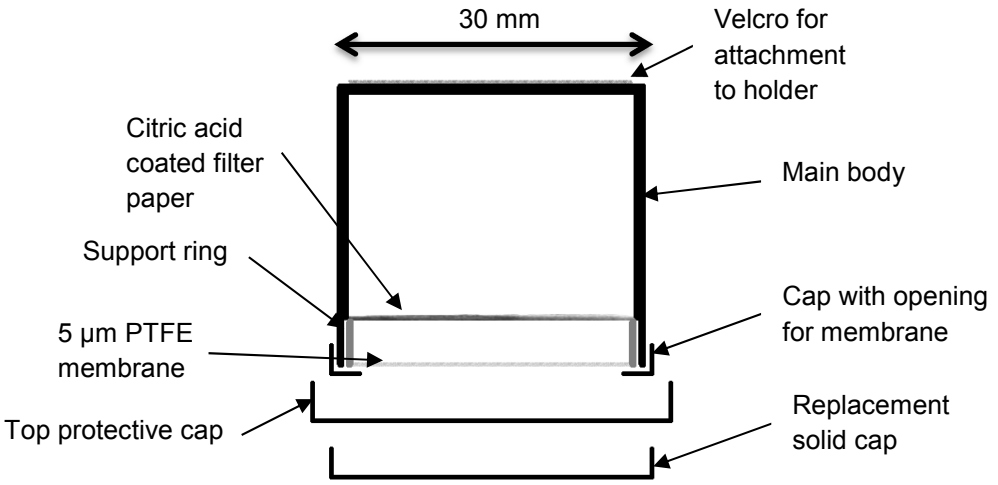


Figure 5.1 Outline diagram of a single ALPHA sampler.

5.2.3 Ammonia determination

Ammonia captured on citric acid coated filter papers was determined by staff at CEH Edinburgh. The citric acid coated filter paper was added to 3 ml deionised water to extract for 1 h. A 1.5 ml aliquot of the resulting solution was placed into a 3 ml auto-sampler tube and the ammonium determined using the Ammonia Flow Injection Analysis system. The system is based on selective

dialysis of ammonium across a membrane at high pH with subsequent analysis of conductivity. Total ammonium was determined using a calibration curve across the range 0 – 10 ppm NH_3 - N.

The final deposition value of ammonia onto the filter paper was determined using Fick's Law of diffusion. The theoretical uptake rate by the ALPHA sampler is a function of the diffusion path length, *L* (m), and the cross sectional area (*A* (m^2)) of the stationary air column within the sampler, and is calculated using the diffusion coefficient, *D* (m^2 s^{-1}) of the gas of interest. Thus, the effective volume of air sampled, *V* (m^3) is given by:

$$V = \frac{DAt}{L}$$

where *t* is the duration of exposure (h).

The air concentration (*X*) of a pollutant is given by:

$$X = \frac{(m_e - m_b)}{V}$$

where m_e is the quantity of NH_3 collected on the exposed citric acid filter paper (µg), and m_b is the quantity of NH_3 in the blank sample (µg).

The mean [NH_3] concentration in air at each site was expressed as µg m^{-3}.

5.2.4 Statistical Methods

SigmaPlot 11 (Systat Software Inc, California, USA) was used to perform standard statistical analyses. All data were subjected to normality and homogeneity of variance tests. If these assumptions were not violated, data were subjected to linear regression analysis or *t*-tests. Non-normal data were log_{10} transformed. Mean [NH_3] values at each site were tested against residual variation in *C. vulgaris* S_W, $[N]_{shoot}$ and $[P]_{shoot}$ and heathland patch size.

Natural or standardised residual variation for the test variables were yielded from the data reported in Chapter 3 of this thesis.

5.3 Results

Mean monthly atmospheric [NH_3] values at the 11 heathland sites studied ranged between 0.96 - 3.50 µg m^{-3}. [NH_3] values varied significantly between monthly measuring dates, with the highest values being observed during March and April 2011 (Figure 5.2).

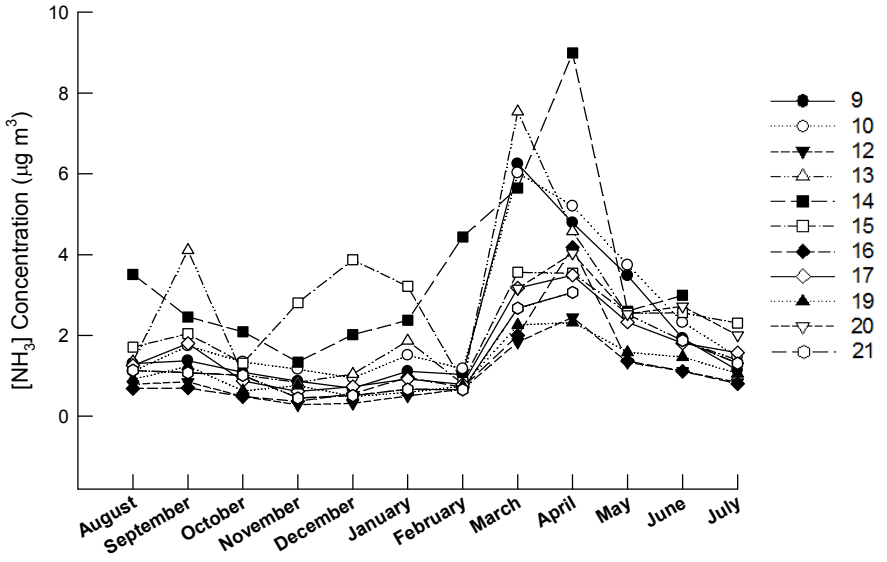


Figure 5.2 Monthly variation in NH_3 concentrations between August 2010 and July 2011 at 11 heathland sites in the East Midlands region of Britain. The numbers in the key represent the site numbers reported in Chapter 2.

There was a significant relationship (r = 0.739, P = 0.009, DF = 10) between the measured mean atmospheric NH_3 concentrations across all sites between August 2010 and July 2011, and the mean 5 x 5 km resolution modelled data for the period 2000 - 2008 (Figure 5.3).

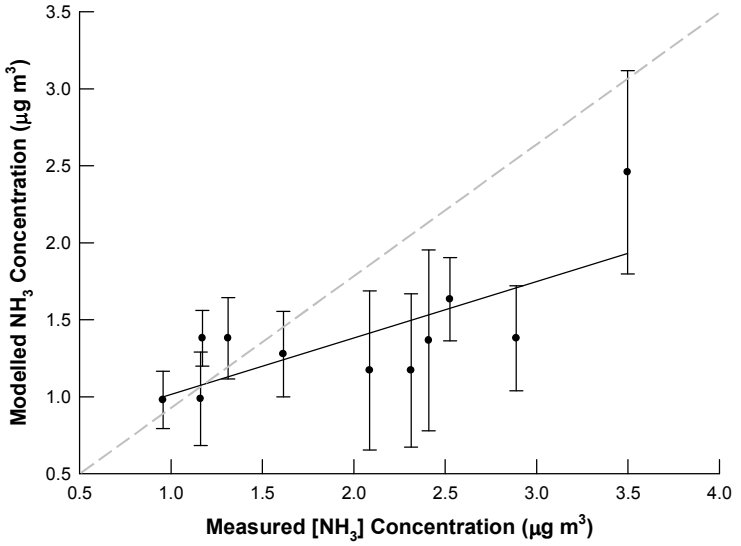


Figure 5.3 Relationship between measured and modelled atmospheric NH_3 concentrations at 11 heathland sites in the East Midlands. Plotted measured data are mean monthly values recorded at 1.5 m above ground level during 2011, and plotted modelled data are mean annual values for the period 2000 - 2008 ± 1 SEM (*n* = 5-12). The solid line represents the linear regression model and the dashed line represents unity.

There were no relationships between mean monthly measured [NH_3] concentrations and the residual variation in S_W (Figure 5.4; r^2 = 0.016, P = 0.713, DF = 10), $[N]_{shoot}$ (Figure 5.5; r^2 = 0.045, P = 0.533, DF = 10), or $[P]_{shoot}$ (Figure 5.6; r^2 = 0.009, P = 0.777, DF = 10) as calculated using the regression models in Chapter 3. Heathland site area was also not related to atmospheric NH_3 concentrations (Figure 5.7; r^2 = 0.120, P = 0.297, DF = 10).

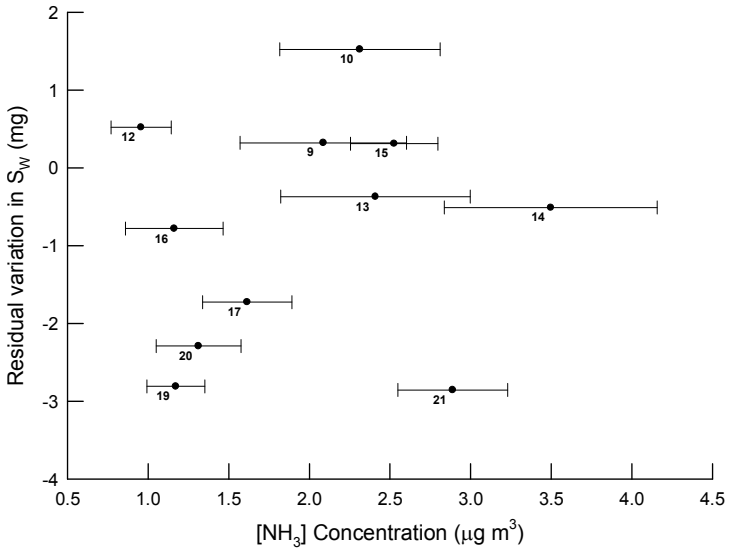


Figure 5.4 Measured [NH_3] values and the residual variation in *Calluna vulgaris* shoot dry-mass in a bioassay using a regression model with N deposition (Figure 3.4a). Plotted values are monthly means (*n* = 5-12) ± 1 SEM.

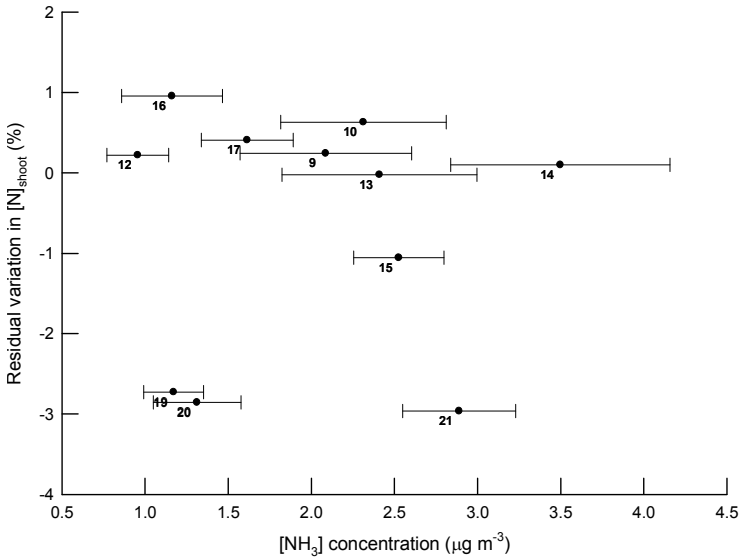


Figure 5.5 Measured [NH_3] values and the residual variation in *Calluna vulgaris* shoot N concentrations in a bioassay using a regression model with N deposition (Figure 3.4b). Plotted values are monthly means (*n* = 5-12) ± 1 SEM.

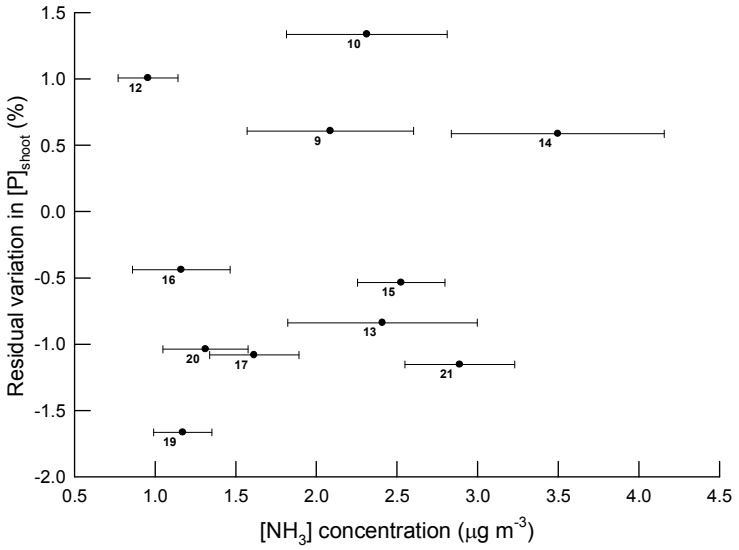


Figure 5.6 Measured [NH_3] values and the residual variation in *Calluna vulgaris* shoot P concentrations in a bioassay using a regression model with N deposition (Figure 3.4c). Plotted values are monthly means (*n* = 5-12) ± 1 SEM.

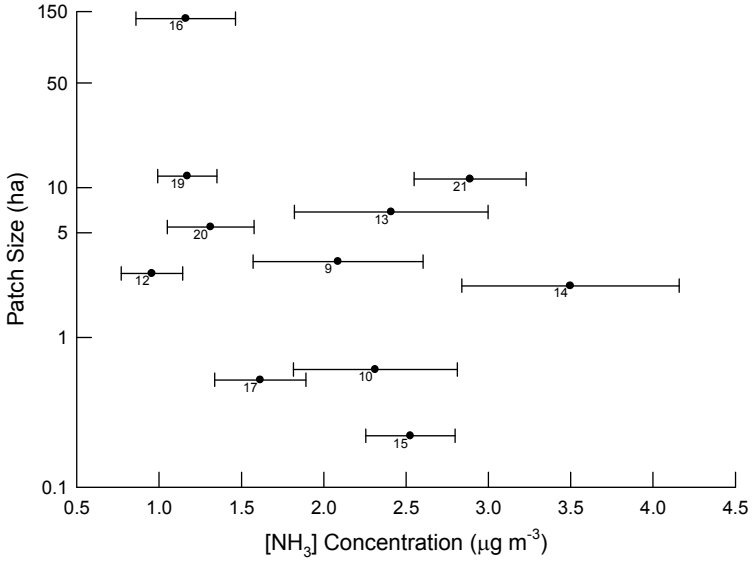


Figure 5.7 Measured [NH_3] values at 1.5 m above ground-level and heathland site area (note log scale). Plotted values are monthly means (*n* = 5-12) ± 1 SEM.

5.4 Discussion

The atmospheric [NH_3] values measured at the 11 heathland sites in this study ranged between 0.96 and 3.50 µg m^{-3}. These values fall into the range of atmospheric NH_3 concentrations data routinely measured at the 80 sites in the UK National Ammonia Monitoring Network (NAMN), i.e. 0.06 to 11 µg m^{-3} (Sutton *et al*., 2001). The 80 monitoring locations in the NAMN were selected to be at least 150 m from any NH_3 emission source, and included known emission peak and trough locations (Sutton *et al*., 1998). Burkhardt *et al*. (1998) recorded similar mean monthly atmospheric ammonia concentrations of 0.7 to 2.0 µg m^{-3} at a rural site in Scotland which was 300 m from an intensive cattle and pig farm. In the present study potentially substantial local ammonia emission sources included a poultry farm near Walesby Forest, cattle farms near Kirkby Moor and Ulverscroft, and a sheep farm near Scotton Common. Mean atmospheric NH_3 concentrations exceeding 2.0 µg m^{-3} were found at Woodhall Spa, Kirkby Moor, Scotton Common, Walesby Forest, Ulverscroft and Vicar Water. All of these sites are close to either a poultry, cattle or sheep farm. The stocking densities of animals varied throughout the year, and there was large variation in the proximity of the farms to the heathlands studied. The emission values for livestock proposed by Misselbrook *et al*. (2000) suggest that the local agricultural practices provided a source of NH_3 to the sites. The remaining sites, with atmospheric NH_3 concentrations within the range 0.95 - 1.61 µg m^{-3}, were all located in urban areas or surrounded by woodland. Hovmand *et al*. (1998) noted that [NH_3] values were markedly reduced over a conifer woodland in Denmark, possibly due to enhanced deposition resulting from lower wind velocity (Dragosits *et al*., 2002). Surrounding woodland could have acted as a buffer to local NH_3 emission sources.

Cape *et al*. (2009) suggest that the critical level for [NH_3] should be 3 ± 1 µg m^{-3} for herbaceous species. This proposed level is much lower than the original value of 8 µg m^{-3} proposed in the UNECE Bad Harzburg workshop in 1993 (Ashmore & Wilson, 1994). The maximum atmospheric [NH_3] value found in the present study was 8.99 µg m^{-3} at Walesby Forest in April 2011. This was the only instance in which [NH_3] values exceeded the 1993 critical level. The proposed lower critical level, however, was exceeded on a number of occasions, particularly during the spring months. The present study found no relationship between [NH_3] values and the residual variation in *C. vulgaris* S_W, or either $[N]_{shoot}$ or $[P]_{shoot}$ seen in Chapter 3. This suggests that, contrary to previous research, measured [NH_3] values in the current study do not explain any additional variance in the bioassay variables. Leith *et al*. (2001) and van der Eerden *et al*. (1991) found that exposing *C. vulgaris* to [NH_3] values in the range 90 to 100 µg m^{-3} resulted in increased foliar N concentrations. Sheppard *et al*. (2011) demonstrated an increase in foliar damage in *C. vulgaris* exposed to NH_3 deposition up to the equivalent of 70 kg N ha^{-1} y^{-1}. There was no significant response of *C. vulgaris,* however, to seven years of NH_3 concentrations up to 4 µg m^{-3}. This suggests that the lack of any significant relationships between atmospheric [NH_3] concentrations and *C. vulgaris* shoot dry-mass and shoot chemistry is because NH_3 deposition onto the heathland sites studied is not enough to significantly increase available soil N. Previous studies have investigated relationships between [NH_3] and foliar N in the field as penetration into the leaves in the primary pathway by which [NH_3] enters plants (Sutton *et al*., 1992; van Hove *et al*., 1987). One would presume that plants with higher foliar N due to [NH_3] enrichment would contribute to soil N via increased leaf litter deposition containing greater N concentrations. This study, however, has found no evidence that this has occurred in the heathland sites studied.

One might expect heathland patches subject to higher [NH_3] to be smaller due to vegetation changes induced by greater N enrichment. This study found no relationship between atmospheric NH_3 concentrations and heathland site area. The placement of the ALPHA sampling device at the sites was frequently affected by restrictions by landowners, or by the presence of grazing animals which might damage the apparatus. In the case of Kirkby Moor, this meant that the ALPHA samplers were placed in close proximity to a cattle farm, 500 m away from the heathland patch. Since NH_3 is deposited close to the emission source due to its low atmospheric residency period (Erisman *et al*., 1988; Ferm, 1998), NH_3 concentrations at the heathland patch may have been lower than those recorded. It is possible, therefore, that signals of NH_3 induced increases in soil fertility may have been confounded by the variation in the distance between the sampling device and the heathland patches from which soils were collected.

This study found no evidence that [NH_3] deposition explained any residual variation in *C. vulgaris* S_W or $[N]_{shoot}$ and $[P]_{shoot}$, and was not related to heathland patch size. There is abundant evidence to suggest that NH_3 increases foliar N concentrations and can result in vegetation composition changes (van den Eerden *et al*., 1991; Leith *et al*., 2001; Sheppard *et al*., 2011). In the present study [NH_3] concentrations ranged between 0.96 and 3.50 µg m^{-3}. Cape *et al*. (2009) suggest that the critical level for the atmospheric concentration of NH_3 should be 3 ± 1 µg m^{-3} for herbaceous species, which is close to the maximum mean value in the current study. It is therefore possible that the [NH_3] concentration values were too low to detect signals of elevated soil fertility across the sites studied, and so do not pose a threat to the sustainability of heathlands subject to NH_3 inputs below 3.50 µg m^{-3}. This finding could serve to inform management of heathlands in close proximity to NH_3 sources within this atmospheric concentration range. The

study of heathlands subject to [NH_3] values greater than 3.50 µg m^{-3} warrants further investigation to discover the impact on soil fertility and vegetation composition.

6

Vegetation Composition of 25 Lowland Heathland Sites across a Nitrogen Deposition Gradient

6.1 Introduction

Nitrogen enrichment has resulted in plant species community changes throughout temperate regions (Bobbink *et al*, 2010; Sala *et al*., 2000). Heathland plant communities are particularly susceptible to N-enrichment due to adaptation to low N availability (Lee, 1998; Lee & Caporn, 1998). As a result, increased N deposition has been identified as a substantial threat to cornerstone heathland plant species, specifically ericoids, and hence habitat sustainability (Aerts & Heil, 1993). The results of the bioassay presented in chapter 3 revealed positive relationships between modelled N deposition and soil fertility, as indicated by *Calluna vulgaris* growth, at the heathland sites studied. The *C. vulgaris* $([N]:[P])_{shoot}$ data suggested that there was no P limitation across the range of N deposition values studied, and that the soil P reserves were sufficient for *C. vulgaris* growth. There were indications that management and heathland patch size modifies the extent to which N deposition affects soil fertility. The present study aimed to discover whether relationships exist between vegetation composition at 25 lowland heathland sites in mainland Britain and the *C. vulgaris* growth and tissue chemistry variables measured in chapter 3, which act as a proxy for soil fertility, environmental factors, management and heathland patch size.

The link between *C. vulgaris* loss and increased soil fertility was first discovered in a series of papers originating in the Netherlands. Heil & Diemont (1983) noticed that in response to repeated artificial applications of N at a rate of 28 kg N ha^{-1} y^{-1}, a heathland community rapidly succeeded into grassland, with *C. vulgaris* being nearly completely replaced by *Festuca ovina* after 12 years of N applications. In chapter 3, it was established that there is a positive relationship between *C. vulgaris* growth and N enrichment. Aerts & Berendse (1988), however, have demonstrated that the graminoid *Molinia caerulea* shows a much greater growth response to N enrichment than the ericaceous species *Erica tetralix*. Consequently, under N enrichment, faster growing species are able to competitively exclude slower growing ericoids, thus ultimately resulting in heathland loss. Increase in bryophyte presence, such as the invasive moss *Campylopus introflexus*, has also been shown to exclude *C. vulgaris* by reducing successful germination by depriving seedlings of light under moss carpets (Equihua & Usher, 1993).

Despite widespread conservation efforts heathlands have become fragmented with area decreasing over recent decades, while the total number of sites has increased partly due to vegetation succession from heathland to scrub, grassland or woodland resulting from inappropriate management, neglect or heavy grazing (Bardgett *et al*., 1995; Rose *et al*., 2000). In Dorset, for example, the number of heathland patches increased from 142 to 151, but the total area decreased from 7925 to 7500 ha between 1978 and 1987 (Webb, 1990). The increased ratio of patch edge to patch area in smaller heathlands may lead to ingress of nutrients from incoming litter, ground water and airborne contaminants from surrounding farmland leading to the encroachment of non-heathland species into the patch and resulting in heathland degradation. Bender *et al*. (1998) for example found that, in a study

of 25 heathland sites, plant species that occur in the interior, such as *C. vulgaris*, are absent in the peripheral zones.

Various management techniques have been implemented in an attempt to mitigate the effect of N enrichment on plant species composition changes. The positive effects of traditional management practices in sustaining heathland vegetation may have been incidental. For example, grazing livestock, such as sheep, cattle or horses, or sod-cutting for fuel had the effect of reducing soil fertility and reducing the presence of undesirable species, such as grasses, and prevented tree encroachment (Heil & Aerts, 1993; Webb, 1986). The vegetation may have also been burnt in order to improve the forage for livestock (Webb, 1998). More recent management techniques, such as mowing and removal of the cuttings, are primarily used to reduce fertility and remove/reduce undesirable plant species, and are frequently used together with the traditional management practices. Attempts have been made to quantify the impact of management techniques on soil fertility. Haerdtle *et al*. (2006) and Mitchell *et al*. (2000) approximated the number of years of atmospheric N input that can potentially be removed by one application of a management technique. Haerdtle *et al*. (2006) found that continuous grazing at low intensities for one year, or one application of prescribed burning can remove around five years of N deposition. Sod-cutting or litter removal, on the other hand, can remove up to the equivalent of 90 and 147 years of N deposition respectively (Haerdtle *et al*., 2006; Mitchell *et al*., 2000). Anecdotal evidence, however, suggests that the impact of management varies between, and indeed within, heathland sites. Burning, for example, can substantially hinder post-fire *C. vulgaris* regeneration if soil moisture was low preceding the burn; soil moisture can potentially vary substantially within a site (Davies *et al*., 2010). Anderson & Radford (1994) showed that reduction in sheep grazing density from 211 to 112 sheep on a 607 ha moorland site in the Peak District

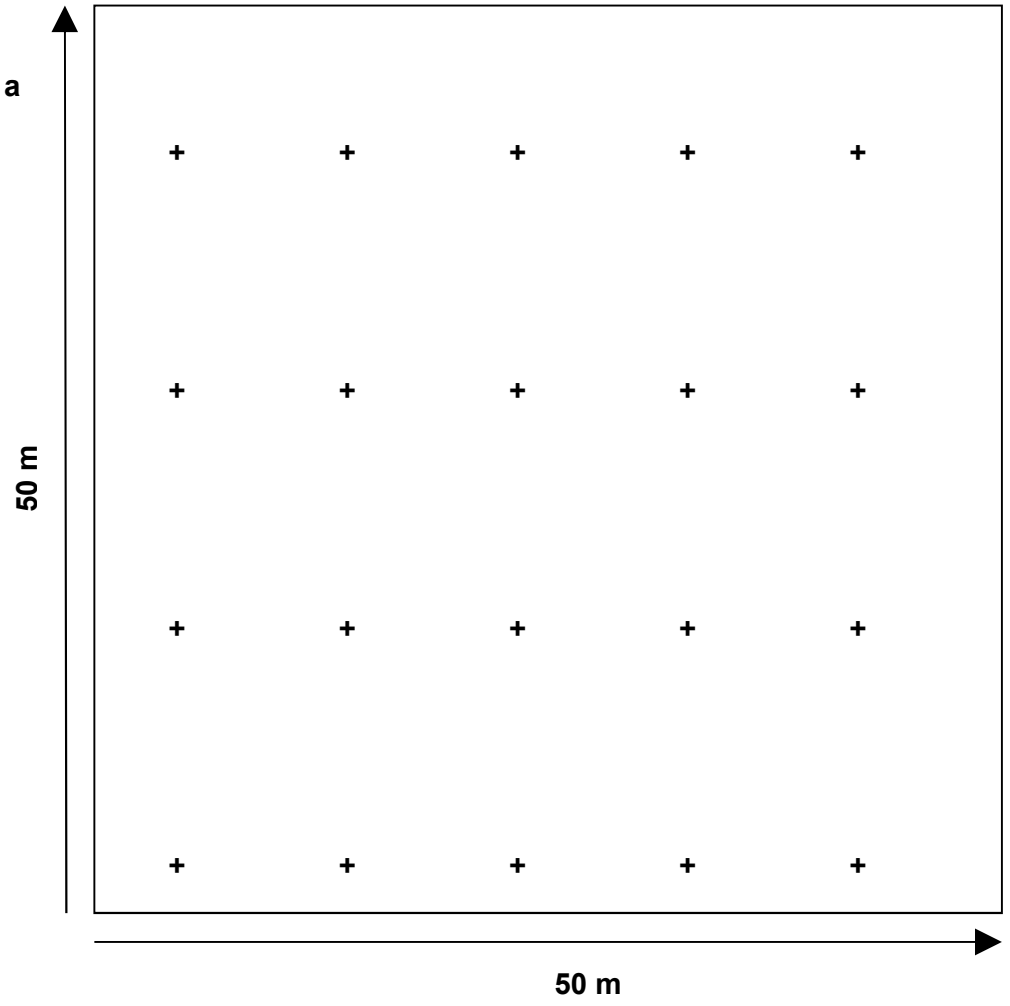
National Park, England, resulted in an increase in *C. vulgaris* from a mean frequency of 1.30 to 8.44% on peaty podzols, and from 0.65 to 31.81% on mineral soils over 17 years, suggesting substantial within site variation in the impact of grazing on vegetation composition.

The link between nutrient enrichment and heathland loss is known, but the evidence has primarily been based on controlled artificial applications of N and the subsequent observations of vegetation composition changes. Management practices are understood to mitigate the impact of N enrichment, but numerous techniques are usually used in unison and their impacts on soil fertility can vary spatially and temporally within and between sites (Davies *et al*., 2010). The combined impact of atmospheric N input, ingress of nutrients from the surrounding matrix, rainfall, geographical location and patch size, and the mitigating impact of management, however, has not been studied. Research is required in order to understand the extent to which these factors are changing heathland vegetation composition. This chapter reports an investigation into the relationships between higher plant, bryophyte and lichen composition and environmental factors such as N deposition, soil fertility, as indicated by the *C. vulgaris* bioassay reported in chapter 3, heathland patch size and geographical location. The effect of management on vegetation composition was also investigated.

6.2 Materials and Methods

6.2.1 Vegetation Survey

Vegetation composition at the 25 low-rainfall lowland heathland sites identified in Chapter 2 was recorded between June and August 2009. A 2500 m^2 sampling plot was selected at each heathland site in an area considered representative of the general vegetation composition. Within this plot twenty 50 x 50 cm quadrats were placed at regular intervals and within each a ten-point 50 cm pin frame (Alana Ecology Ltd, Shropshire, UK) was deployed five times to obtain 50 data points per sample, and a total of 1000 per site (Figure 6.1). Top cover was determined by vertically lowering a pin and recording the first individual that the point touched (Causton, 1988; Greig-Smith, 1983). A presence-absence record of all species within the larger quadrat was also collected. In all cases vascular plant, bryophyte and lichen species were recorded. Identifications were based on Rose (1989; 2006) and Jermy *et al*. (2007) for flowering plants, grasses, sedges rushes and ferns, Smith (2004) and Watson (1981) for bryophytes, and Dobson (2005) for lichens.



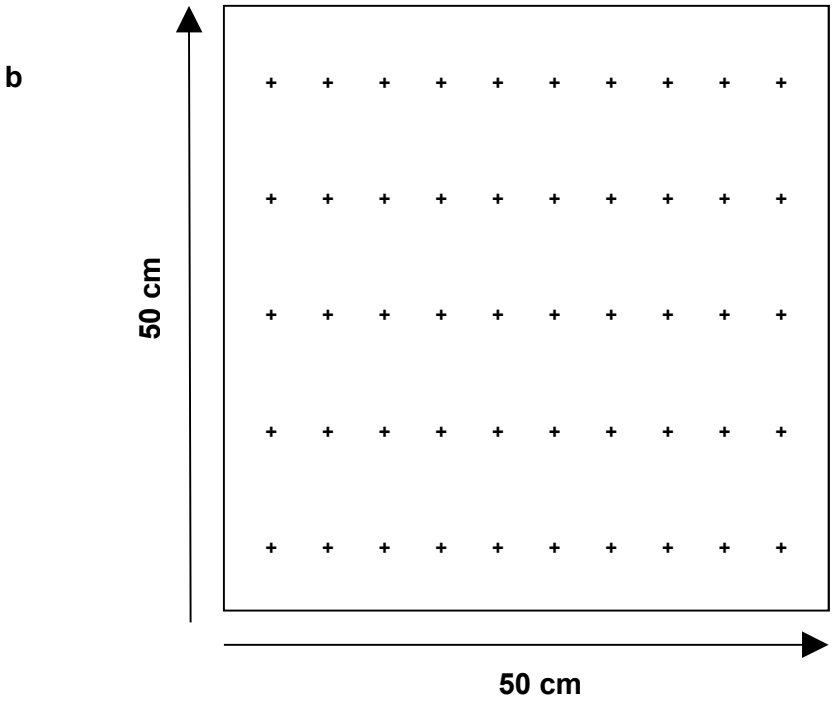


Figure 6.1 Example of sampling design for (a) 2500 m^2 plot, and (b) 50 x 50 cm quadrat.

6.2.2 Statistical Methods

Species diversity and estimated richness indices for each site were calculated using the Species Diversity and Richness v.4.1.2 package (Pisces Conservation Ltd, Hampshire, UK). Chao's quantitative estimator of species richness was used to extrapolate minimum species richness (Chao, 1984). Chao's estimator presents the absolute number of species in an assemblage, based on the number of rare species in a sample (Magurran, 2004). Simpson's *D* (1/*D*) and *E* ($E_{1/D}$) indices (Simpson, 1949) and Shannon's *H* ($_{exp}H$) index (Shannon & Weaver, 1949) were used to calculate species diversity and evenness at each site. Simpson's *D* describes the probability that any two individuals drawn at random from an infinitely large community belong to the same species (Magurran, 2004). Higher 1/*D* and $_{exp}H$ values indicate greater species diversity (Kent & Coker, 1992). The Simpson's *E* value ranges from 0 to 1, with a higher value indicating greater species evenness.

Vegetation composition data were analysed using the non-metric multidimensional scaling (NMDS) method in Community Analysis Package v.4.1.3 (Pisces Conservation Ltd., Hampshire, UK). Environmental variables were overlain as vectors on a Canonical Correspondence Analysis (CCA) ordination in Ecological Community Analysis v.2.1.3 package (Pisces Conservation Ltd., Hampshire UK). The environmental variables included for each site were N_W, $[N]_{shoot}$ and $[P]_{shoot}$, and S_W of, *C. vulgaris* in the bioassay (Chapter 2.2.1), latitude and longitude, rainfall and patch size. The two axes yielded by the NMDS ordination were also included as two predictors.

Multiple GLM models were conducted on the two NMDS axes and the diversity and richness indices using the explanatory environmental variables listed above. A model simplification procedure (<0.05 for inclusion) was used

to build each model. The significance of each variable was tested based on deviance change upon removal from the full model. All GLM models were built in R v.2.11.0 (R Foundation for Statistical Computing, Vienna, Austria).

6.3 Results

6.3.1 Species richness and diversity

A total of 77 plant and lichen species were recorded across all sites, of which 8 were ericaceous shrubs, 27 were graminoids, 17 were forbs or ferns, 5 were trees, 15 were bryophytes, and 5 were lichens (Table 6.1). Bare ground comprised 2.6% of all points. Species accumulation curves indicated that sampling was complete for 11 of the 25 sites. Estimated species richness, Simpson's D and E and Shannon's H diversity indices are presented in Table 6.2. Species richness ranged from 3.0 ± 1.12 to 34.0 ± 15.17, Simpson's D and E indices ranged from 1.02 to 5.42 and 0.13 to 0.50, respectively. Shannon's H diversity index ranged from 1.05 to 6.90.

6.3.2 Species composition

An NMDS ordination achieved a solution with two dimensions (Figure 6.2). Vectors of environmental variables in the CCA indicate a strong relationship with Axis 1 of the NMDS ordination (Figure 6.3).

Table 6.1 Complete list of plant and lichen species found during quadrat sampling for all heathland patches.

Ericaceous shrubs	*Calluna vulgaris*
	Erica cinerea
	E. tetralix
	E. vagans
	Ulex europaeus
	U. gallii
	Vaccinium myrtillus
	V. uliginosum
Graminoids	*Agrostis canina*
	A. capillaris
	A. gigantea
	A. stolonifera
	Anthoxanthum odoratum
	Blysmus compressus
	Carex nigra
	C. panicea
	Danthonia decumbens
	Deschampsia flexuosa
	D. setacea
	Eleocharis uniglumis
	Festuca ovina
	F. pratensis
	F. rubra
	Holcus lanatus
	H. mollis
	Juncus conglomeratus
	J. effusus
	J. squarrosus
	Luzula campestris
	L. multiflora
	L. pilosa
	Molinia caerulea
	Nardus stricta
	Poa nemoralis
	Schoenus nigricans
Forbs and ferns	*Centaurea nigra*
	Chamerion angustifolium
	Cirsium vulgare
	Cytisus scoparius
	Drosera rotundifolia
	Dryopteris filix-mas
	Empetrum nigrum
	Eriophorum angustifolium
	Galium saxatile
	Hypochaeris radicata
	Ornithopus perpusillus
	Pedicularis sylvatica
	Pteridium aquilinum
	Ranunculus repens
	Rubus fruticosus agg.
	Rumex acetosella
	Teucrium scorodonia
Trees	*Betula pendula*

	Frangula alnus
	Pinus sylvestris
	Quercus robur
	Salix repens
Bryophytes	*Campylopus flexuosus*
	C. introflexus
	C. pyriformis
	Dicranum scoparium
	Eurhynchium praelongum
	Hylocominum splendens
	Hypnum cupressiforme var. lacunosum
	H. cupressiforme variant
	H. jutlandicum
	Polytrichum commune
	Pseudoscleropodium purum
	Sphagnum cuspidatum
	S. palustre
	S. subnitens
Lichens	*Cladonia chlorophaea*
	C. floerkeana
	C. portentosa
	C. squamosa
	C. uncialis
Other	Bare Ground
	Non-identifiable (agricultural escape)

Table 6.2 Comparison of different richness and diversity indices for the 25 heathland study sites.

Site	Estimated Species Richness	Shannon's H	Simpson's D	Simpson's E
Heathmount North	30.5 ± 11.56	4.21	2.47	0.14
Hunting Hill	13.0 ± 1.58	2.53	1.60	0.13
Lundy Island	10.3 ± 0.61	2.12	1.50	0.15
Goonhilly Downs	12.5 ± 1.12	4.87	2.88	0.24
Coverack	13.0 ± 3.01	5.21	4.13	0.38
Ashdown Forest	6.5 ± 1.12	3.41	3.00	0.50
Hothfield Common	11.3 ± 0.61	2.63	2.11	0.19
Horsell Common	5.0 ± 0.00	2.72	2.29	0.46
Woodhall Spa	7.0 ± 1.17	2.39	1.83	0.26
Kirkby Moor	34.0 ± 15.17	2.22	1.49	0.09
Skipwith Common	13.0 ± 1.58	2.77	1.91	0.16
Clumber Park	19.5 ± 5.44	5.60	4.23	0.28
Scotton Common	14.0 ± 3.01	4.13	3.27	0.25
Walesby Forest	7.5 ± 1.12	3.11	2.61	0.37
Ulverscroft	19.3 ± 2.81	6.90	5.42	0.32
Budby Heath	15.0 ± 1.07	4.86	3.53	0.24
Beacon Hill I	15.5 ± 1.12	4.25	2.41	0.16
Beacon Hill II	9.0 ± 1.12	4.51	3.47	0.39
Woolley Moor	8.0 ± 1.12	3.12	2.37	0.26
Sherwood Forest	12.5 ± 1.12	4.46	3.33	0.28
Oak Tree Heath	10.0 ± 3.01	2.57	2.00	0.25
Vicar Water	14.0 ± 0.01	3.15	1.98	0.15
Brizlee Wood	8.5 ± 1.12	1.50	1.18	0.15
Thimbleby Moor	3.0 ± 1.12	1.05	1.02	0.34
Litcham Common	14.0 ± 0.97	2.85	1.78	0.13
Holt Lowes	12.3 ± 0.61	5.84	4.67	0.39

6.3.3 Effect of environmental variables on species richness, diversity and composition

There was no relationship between *C. vulgaris* percentage cover (Figure 6.4; r^2 <0.01, P = 0.053, DF = 25; r^2 = 0.02, P = 0.842, DF = 25) or graminoid percentage cover (Figure 6.5; r^2 = 0.027, P = 0.417, DF = 25; r^2 = 0.02, P = 0.447, DF = 25) and N_W deposition or S_W, respectively.

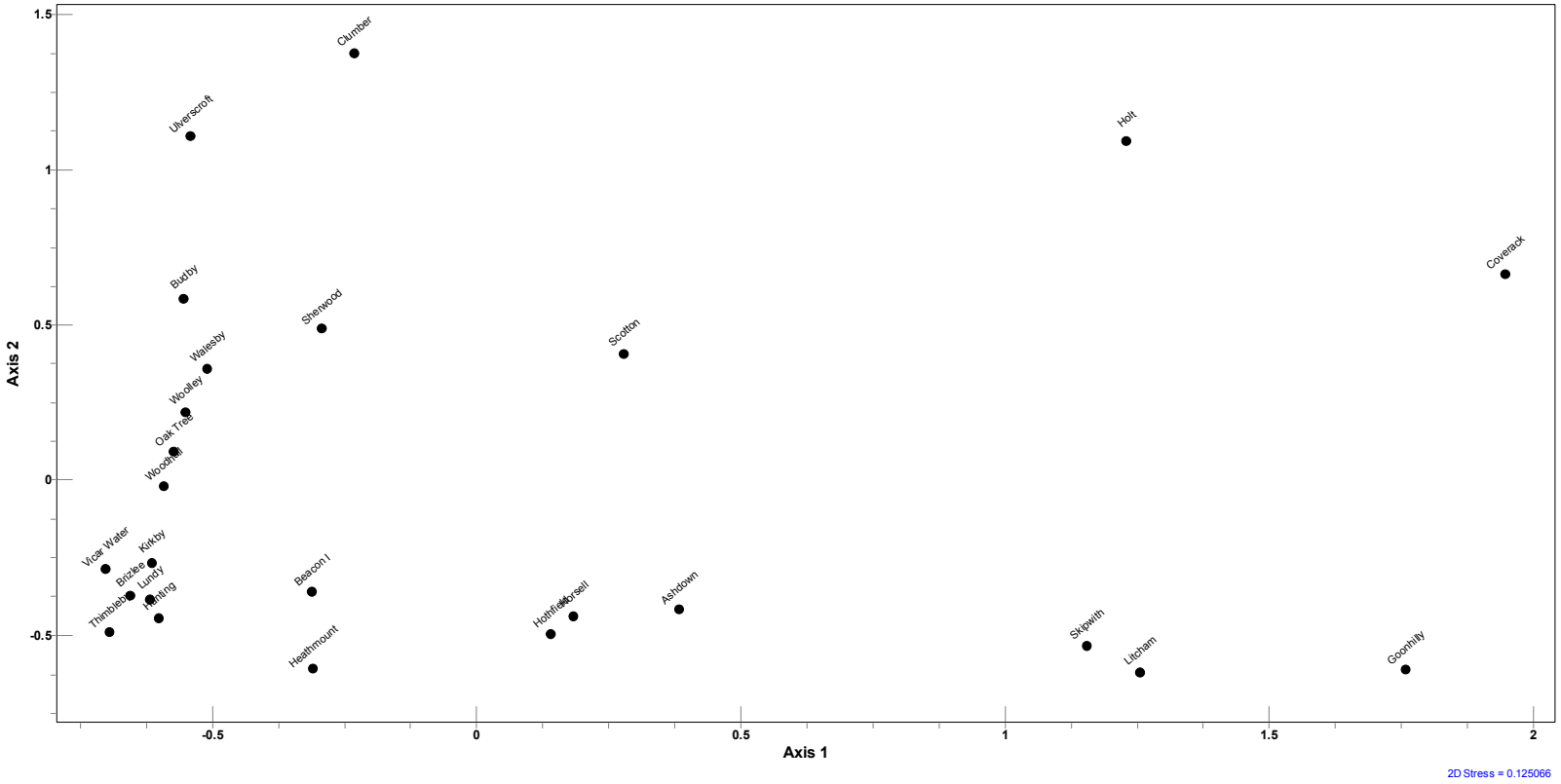


Figure 6.2 A rotated NMDS ordination of the 25 study sites using Bray-Curtis similarity measures. The 2D stress value was 0.125.

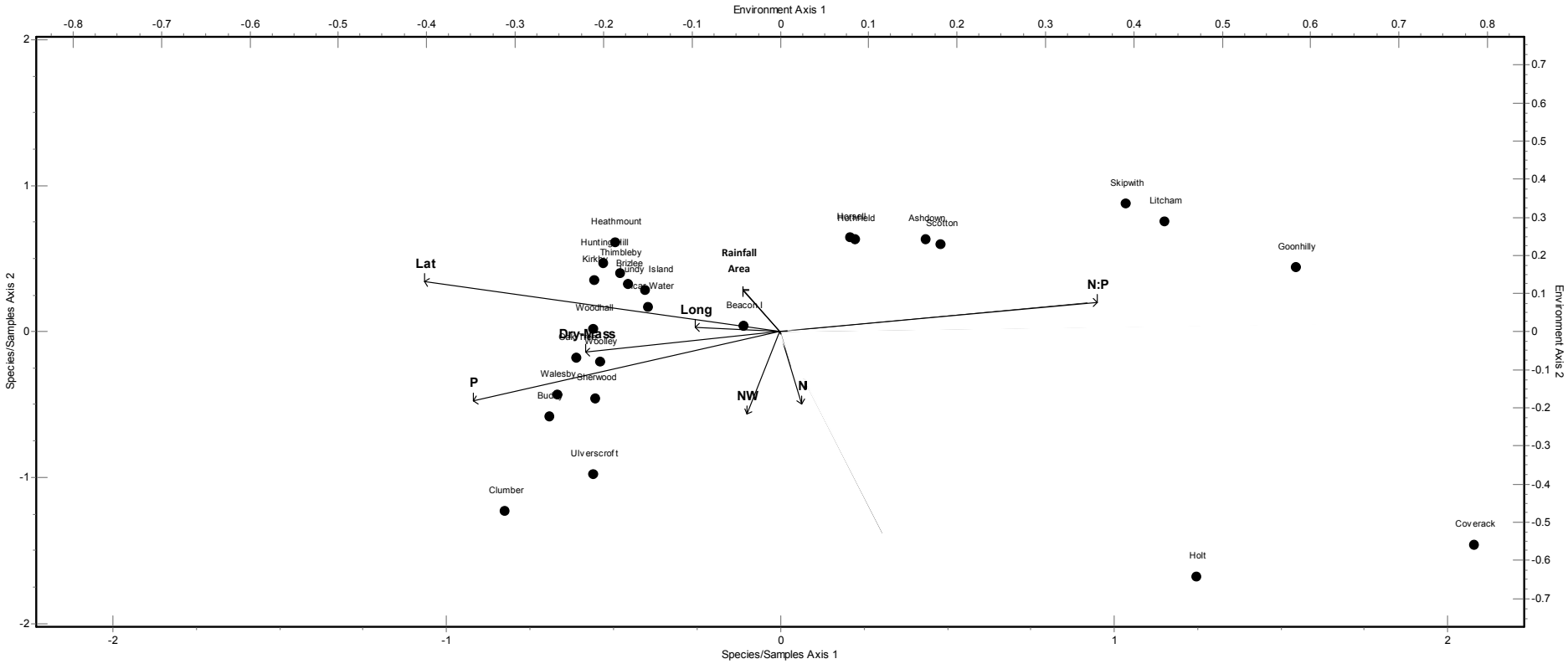


Figure 6.3 CCA ordination of the 25 study sites with the environmental variables overlain as vectors. The length of each vector is proportional to the power of the relationship between the environmental variables and the vegetation composition.

The coefficients of the variables selected for multiple GLMs are presented in Table 6.3. Neither N_W or N_D could explain any variation in vegetation composition, species diversity or richness. $[P]_{shoot}$ was positively and significantly related to Axis 1 of the NMDS ordination (Table 6.3).

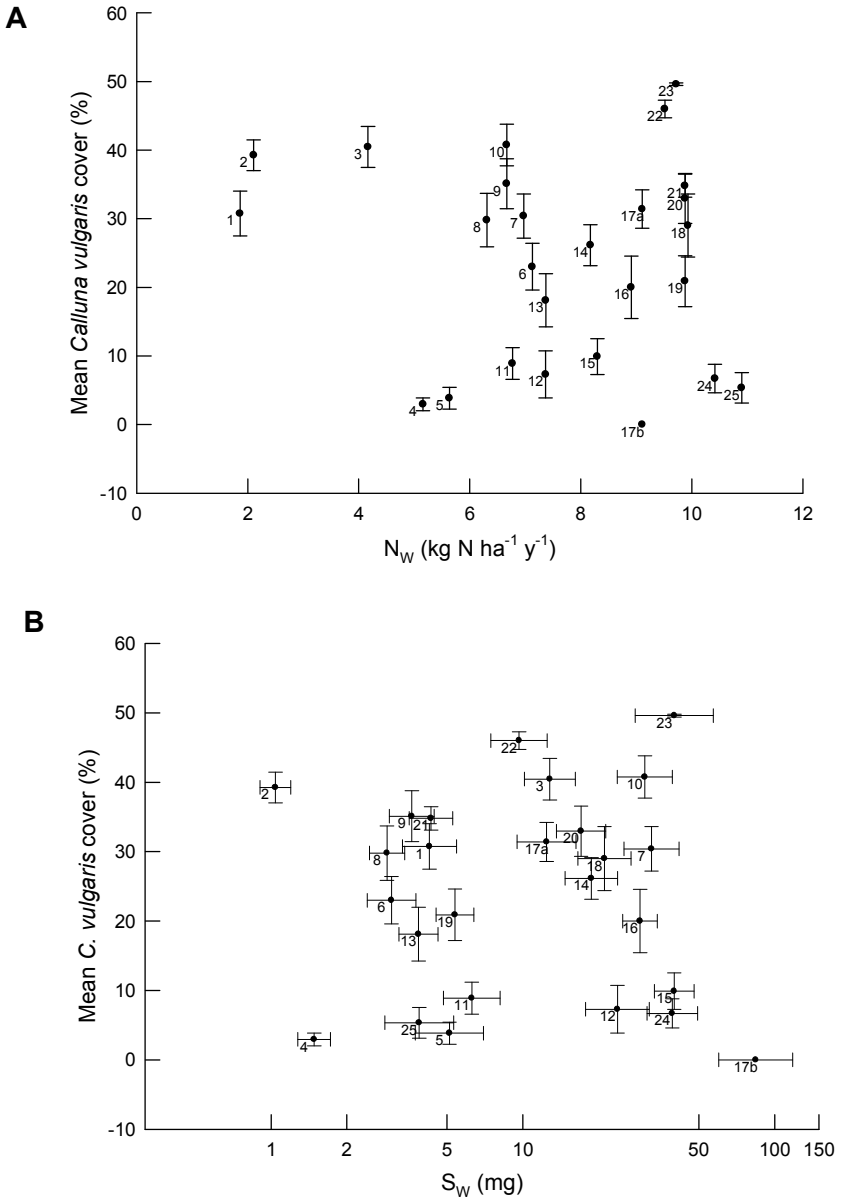


Figure 6.4 Relationship between *C. vulgaris* cover and (A) N_W, and (B) *C. vulgaris* shoot dry-mass (note the log scale) at the 25 heathland sites. Plotted values are means for each site (n = 20) ± 1 SEM. The numbers correspond to the site numbers reported in Table 2.1).

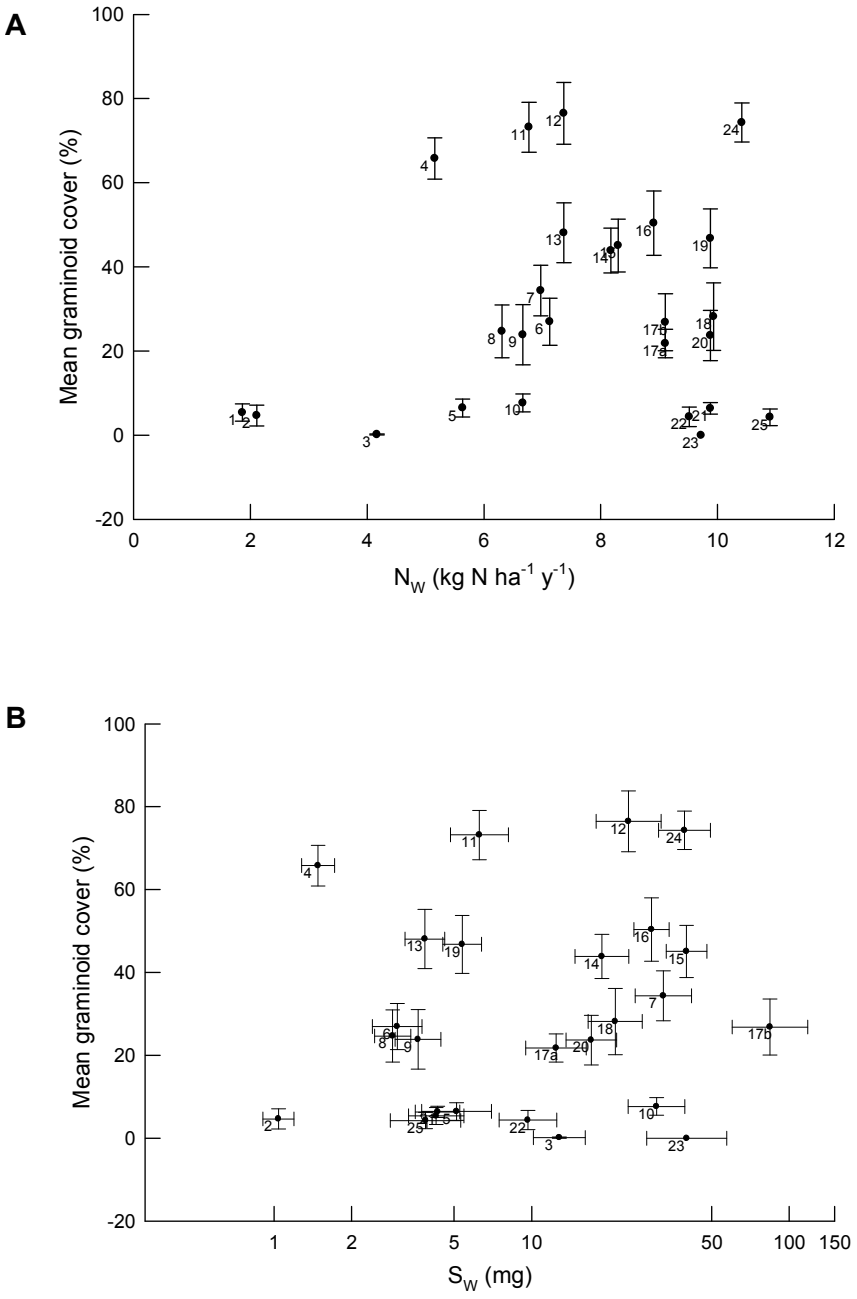


Figure 6.5 Relationship between graminoid (grass, sedge and rush) cover and (A) N_W, and (B) *C. vulgaris* shoot dry-mass (not log scale) at the 25 heathland sites. Plotted values are means for each site (n = 20) ± 1 SEM.

The GLM revealed that species richness was positively related to $[P]_{shoot}$, rainfall and NH_3 in combination (Table 6.3). None of these variables were related to species richness when analysed individually (Figure 6.6).

Table 6.3 Summary of minimum optimal GLMs for NMDS axes, species richness, diversity and evenness. Total wet (N_W) and total dry (N_D) N deposition and NH_3 concentrations were tested against rainfall, latitude, longitude and patch size (log_{10} transformed) and *C. vulgaris* yield and chemistry. For all variables *DF* = 25.

		Parameter Estimates		Model Building Results		
Variable	**Optimal Model**	**Estimate (± 1 SE)**	***P* value**	**AIC**	***P* value**	r^2
Axis 1				55.94	0.03	0.189
	P concentration	-6.69 (2.88)	0.03			
Axis 2	-	-	-	-	-	-
Shannon's H	-	-	-	-	-	-
Simpson's D	-	-	-	-	-	-
Simpson's E	-	-	-	-	-	-
Species Richness				166.57	0.03	0.349
	P concentration	65.06 (27.36)	0.03 0.02			
	Rainfall	-0.03 (0.013)	0.04			
	NH_3	-5.98 (2.28)				

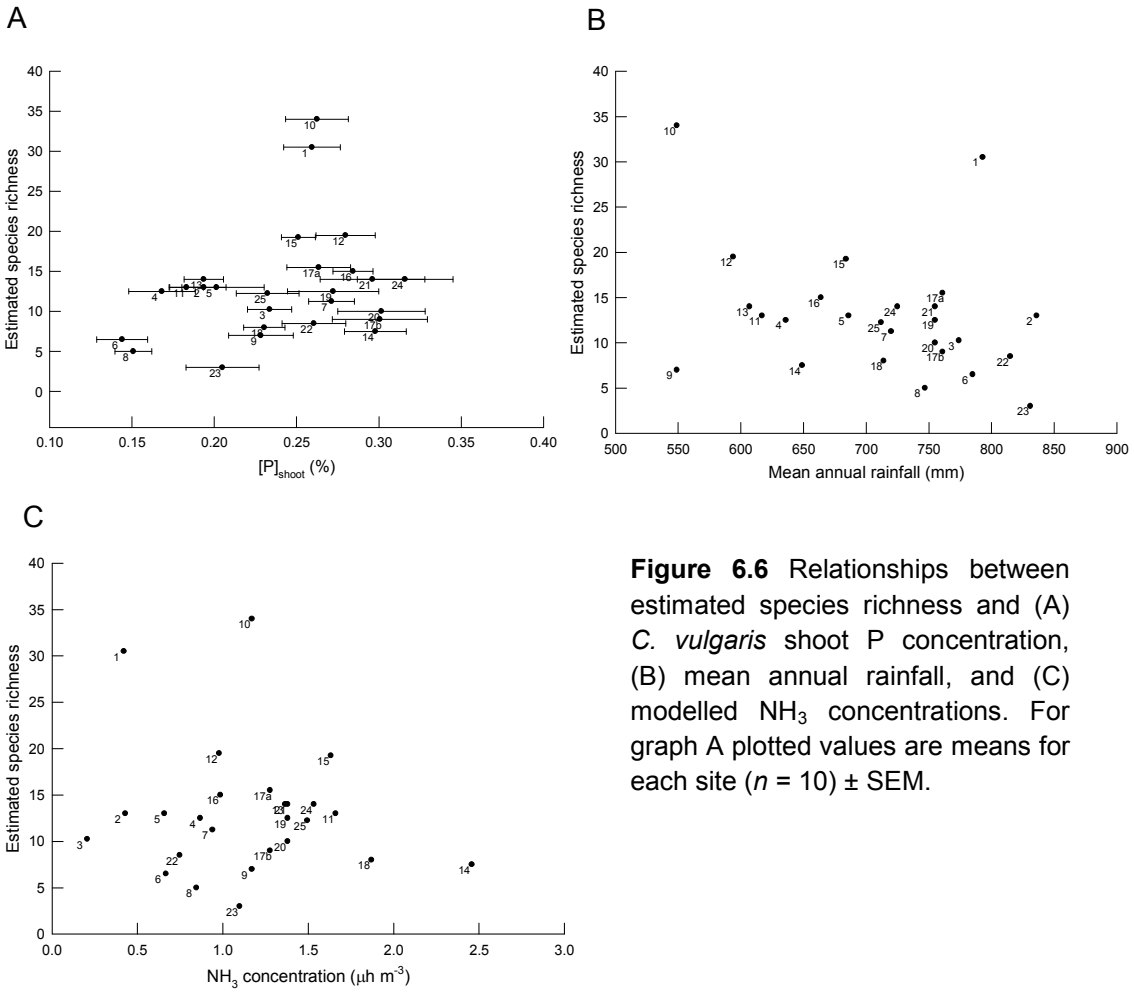


Figure 6.6 Relationships between estimated species richness and (A) *C. vulgaris* shoot P concentration, (B) mean annual rainfall, and (C) modelled NH_3 concentrations. For graph A plotted values are means for each site (n = 10) ± SEM.

6.4 Discussion

Shannon's or Simpson's species diversity indices across the 25 heathland study sites were not significantly related to any variable tested. The GLM revealed that species richness was positively related to $[P]_{shoot}$ in *C. vulgaris*, annual rainfall and modelled NH_3 values. In a study of 68 acid grasslands in the UK, covering an N deposition range of 5 to 35 kg N ha^{-1} y^{-1} Stevens *et al*. (2004) found a strong negative relationship (r^2 = 0.55) between species richness and total N deposition. Similar relationships were found by Maskell *et al*. (2010) in heathland (r^2 = 0.17) and acid grassland (r^2 = 0.09), but not

calcareous grassland (r^2 = 0.005). Stevens *et al*. (2004) noted that annual rainfall explained 8% of the variation in species richness, a relationship that is corroborated by the current study. The relationship between species richness and $[P]_{shoot}$, which is used here as a proxy for P availability, could be indicative of species response to low P availability. Kirkham (2001) and Roem *et al* (2002), for example, found that *Molinia caerulea,* is better adapted to coping with low P situations than ericoids and could outcompete ericoids in a low P system.

This study found no relationship between patch size and any variable tested. In chapter 3 it was found that heathland patch size was weakly negatively related to *C. vulgaris* S_W suggesting a trend for increasing soil fertility within smaller patches. There was, however, no evidence that this translated into a change in vegetation composition. The increased ratio of patch edge to patch size in smaller heathlands may promote ingress of nutrients from incoming litter from the surrounding vegetation, ground water, and dust from farmland, potentially changing the vegetation composition at the patch edge. Piessens *et al*. (2006) reported an increase in soil fertility penetrating 8 m into heathland patches adjacent to cropland and woodland, and found that this substantially increased graminoid dominance, and reduced the presence of ericoids, particularly when the site was adjacent to cropland. Dieckhoff *et al.* (2006) report that plant species richness increased with patch size at ten heathland sites in Germany, but was also positively related to soil pH and moisture. The current study did not sample the entire heathland area at each site, but sampled a 2500 m^2 area irrespective of the patch size. Therefore it is possible that vegetation change caused by ingress of nutrients from surrounding areas may have been detected in peripheral regions of smaller heathland patches sampled in the current study.

This investigation has indicated that factors that vary on a large scale, such as N deposition, are not related to vegetation composition at 25 heathland sites in Britain. During the vegetation surveys conducted for the current study cursory observations suggested that management techniques, such as burning or sod-cutting, had a strong influence on vegetation composition. These effects, however, were not possible to quantify using the data available. Some records of management were incomplete and management regimes varied markedly between sites both spatially and temporally. Even where management records were complete, research has indicated that management impacts on vegetation composition are dependent on many factors. In a study of the effect of grazing by 1500 to 3000 sheep on a heathland in Scotland, Alonso *et al*. (2001) demonstrated that even with uniform grazing, the impact on plant species composition varied substantially within the patch. *Nardus stricta*, for example appeared to outcompete ericaceous species when soil moisture was high, nutrient availability low, and grazing intensive. *Deschampsia flexuosa,* on the other hand, was successful if grazing density was low, and soil moisture was high. The modifying effect of management practices on vegetation composition is well known (see Chapter 1). Given the substantial body of research suggesting that increased nutrient availability negatively affects plant species richness and composition in a range of habitats, the lack of a relationship between N_W and species composition in the present study points to the likely mitigating effects of local factors, such as management intervention.

Previous chapters have found that there is a positive relationship between N deposition and soil fertility in lowland heathlands. Evidence from artificial applications of N suggests that this increase in fertility leads to vegetation composition change, graminoid invasion, and ultimately heathland loss. The

current study indicates that these results do not necessarily transfer to field situations possibly due to complex interactions between large-scale and local variables, including N deposition, rainfall, geographical position, geology, and management regimes. The latter are generally targeted to address site-specific problems, both temporal and spatial, and it is likely that management is a major contributor to the disparity between N induced increases in soil fertility, and the apparent lack of vegetation response at the heathland study sites.

7

The Response of *Calluna vulgaris* Growth to Temperature Increase: The Effect of Temperature at Origin

7.1 Introduction

The current distribution of *Calluna vulgaris* is wide, occurring from Morocco to Scandinavia, and from the Azores to the Urals (Figure 7.1; Loidi *et al*., 2010). Loidi *et al*. (2010) provide the most recent distribution map of *C. vulgaris* dominated heathland, but it must be noted that the range of *C. vulgaris* extends further north into Norway and Finland, and further East to Italy than shown here.

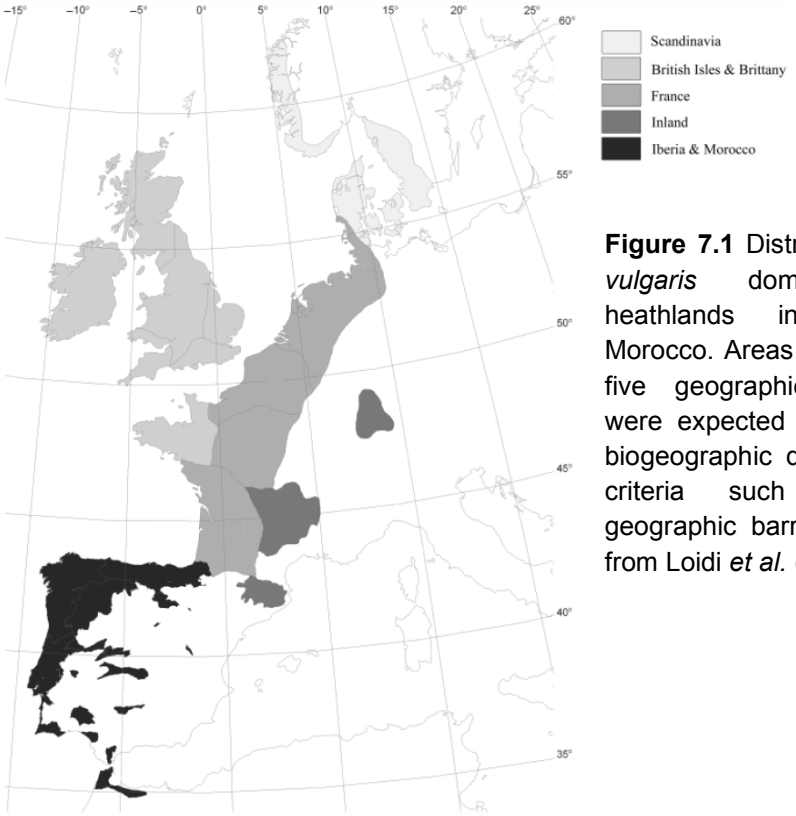


Figure 7.1 Distribution map of *C. vulgaris* dominated lowland heathlands in Europe and Morocco. Areas are classified into five geographic groups which were expected to express broad biogeographic diversity based on criteria such as insularity, geographic barriers *etc*. Modified from Loidi *et al.* (2010).

As described in previous chapters, the current project focuses on lowland heathlands in low rainfall temperate climates. *Calluna vulgaris* however grows in a wide range of soil types, climates and altitudes (Gimingham, 1960). Loidi *et al.* (2010) suggest that the distribution of heathlands is primarily limited by low temperatures in the northern extent of its range, and by summer droughts in the southern extent.

The genetic diversity of *C. vulgaris* across its extensive range was described by Rendell & Ennos (2002) using chloroplast DNA. It was determined that *C. vulgaris* populations varied genetically both within and between populations, with greater diversity located in Southern Europe, as opposed to Northern Europe. This was ascribed to long distance seed dispersal potential being greater in the windier treeless environments in Northern countries, such as Norway and Sweden. On the other hand Mahy *et al*. (1997) found similar trends in genetic diversity, but ascribed lower diversity in the North to the glacial and post-glacial history of *C. vulgaris*, including the locations of glacial refugia. In a study of genetic variation between *C. vulgaris* from heathland sites across Great Britain, Meikle *et al*. (1999) found a significant positive correlation (r = 0.618) between genetic distance and geographical distance, suggesting that populations migrated to the current location and subsequently differentiated *in situ*.

In 1978, Bannister (1978) reported a negative relationship (r = -0.84) between the shoot extension in *C. vulgaris* originating from different geographical locations in Britain when grown in a common garden in Scotland, and temperature at origin. Bannister (1978) found that increasing temperature at origin resulted in lower shoot extension, and delayed flowering in three ericaceous species. Murtagh *et al*. (2002) reported consistent and significant negative relationships (r^2 = 0.784 - 0.815) between the relative growth rate

(RGR) of the lichen-forming fungus, *Xanthoria elegans,* in axenic culture and the temperature at origin, when grown at 2, 5, 12, 18 or 26 °C. The RGR decreased in all cases, except at 26°C, which may have been supra-optimal; but even at this temperature the negative relationship between RGR and temperature at site of origin was maintained.

It is predicted that by 2099 mean annual temperatures will increase by between 2.3°C and 5.3°C (IPCC, 2007). Peñuelas *et al*. (2004) reported that a 1 °C two-year increase in temperature resulted in a 15% increase in above ground plant biomass at an ericoid dominated shrubland in the UK. Changes in temperature affect many ecosystem processes, including litter decomposition rates, nitrification and denitrification, and nutrient uptake, photosynthetic performance and growth in plants (Llorens *et al*., 2004; Rustad *et al*., 2001; Schmidt *et al*., 2004; Wessel *et al*., 2004; White *et al*., 1996). Increased plant growth can be detrimental to heathlands because species with higher RGR values than *C. vulgaris*, such as *Pteridium aquilinum* (L. Kuhn), will grow considerably faster under increased temperatures (Werkman & Callaghan, 1999). This could result in a reduction in *C. vulgaris* cover due to increased shading by *P. aquilinum* (Werkman & Callaghan, 1999), ultimately contributing to a contraction or migration of the effective range of *C. vulgaris* at its latitudinal extents. The impacts of climate on species ranges has often been assessed using bioclimatic envelope models (see Heikkinen *et al*., 2006). These models have been successful in projecting the direction of climate change-driven range changes, but less effective in estimating the magnitude of such changes (Araújo *et al*., 2005). A model by Araújo *et al*. (2011) of the climatic suitability of 1883 European animal and plant species located in conservation areas predicted that 58 ± 2.3 % of species would

experience decreases in their climatic envelopes by 2080, effectively leading to reductions in their ranges.

Given the extensive latitudinal range of *C. vulgaris*-dominated heathlands occurring across a variety of temperatures, and evidence to suggest genetic variation within and among populations, it seems reasonable to suggest that individuals in different populations will exhibit different growth characteristics under contrasting climatic conditions. With temperatures predicted to increase, and some plant species expected to lose areas of climatic suitability, there is a need to discover the possible response of *C. vulgaris* under a climate change scenario to investigate the impact of temperature on growth, and to discover the possibility of a migration in its current range extent.

This chapter reports an investigation into the relationship between the growth of *C. vulgaris* sourced from sites across a latitudinal gradient in Europe, and the annual mean (TO_{mean}), minimum (TO_{min}) and maximum (TO_{max}) temperatures and annual rainfall values at the origin site. *Calluna vulgaris* individuals were grown at a range of temperatures to investigate the W_W, S_W, R_W and R_W:S_W response. The objective of this study was to explore the possibility and extent of local adaptation of *C. vulgaris* to temperature.

7.2 Materials and Methods

7.2.1 Collection of Calluna vulgaris seed

Calluna vulgaris seeds were collected from 21 sites during Autumn 2010 across a latitudinal gradient in western Europe extending from 38° N to 70° N and from 8° W to 27° E (Table 7.1). Annual mean, minimum and maximum monthly temperature values, and mean annual rainfall data for 1901 to 2006 at a 5° gridded square scale were sourced from the University of East Anglia

Table 7.1 Details of sites from which *C. vulgaris* seeds were collected. Temperature and rainfall data are provided as annual mean values for the period 1901 to 2006 (University of East Anglia Climatic Research Unit).

Site Number	Collection Site	Collector	Latitude (°N)	Longitude	Mean Annual Temperature (°C)	Mean Minimum Temperture (°C)	Mean Maximum Temperature (°C)	Mean Annual Precipitation (mm)
1	Kevo Strict NR Finland	Ilkka Syvänperä University of Turku	69.45	27.00°E	-2.33	-7.01	2.34	453.69
2	Skogsfjord Norway	Elisabeth Cooper University of Tromsø	69.94	19.28°E	-0.36	-3.39	2.66	934.28
3	Reble Nesoya Norway	Elisabeth Cooper University of Tromsø	70.00	18.80°E	0.91	-1.99	3.79	970.05
*4	Asikkala Finland	Minna-Maarit Kytöviita University of Jyväskylä	61.13	25.59 E	3.67	-0.22	7.59	583.08
*5	Trondheim Norway	Kari Austnes Norwegian Institute for Water Research	69.23	10.28°E	3.86	0.69	7.02	991.21
6	Blestolen Norway	Kari Austnes Norwegian Institute for Water Research	59.46	9.27°E	4.93	1.37	8.48	1001.81
*7	Remøya Norway	Kari Austnes Norwegian Institute for Water Research	62.37	5.64°E	5.39	2.60	8.20	1613.56
*8	Langesund Norway	Inger Elisabeth Måren University of Bergen	59.01	9.43°E	6.50	3.05	17.60	1055.97
*9	Bergen Norway	Inger Elisabeth Måren University of Bergen	60.28	5.16°E	7.11	4.51	9.74	2071.88
10	Faster Denmark	Sara Alstrup University of Copenhagen	55.98	8.60°E	7.91	4.76	11.09	808.44

*11	Lüneburger Heide Germany	Maren Meyer-Grünefeldt University of Lüneburg	53.18	9.93°E	8.71	4.99	12.44	718.72
*12	Nemitzer Heide Germany	Werner Haerdtle University of Lüneburg	53.97	11.36°E	9.01	5.39	12.70	565.52
*13	Navazo Spain	Peter Crittenden University of Nottingham	40.24	1.24°W	9.08	3.07	15.15	481.61
*14	Beacon Hill England	Peter Crittenden University of Nottingham	52.42	1.17°W	9.26	5.11	13.43	610.47
*15	Ponga Spain	Leonor Calvo Luz Buena University of León	43.24	5.24°W	9.27	5.18	13.44	956.26
*16	Cestas France	Jean Timbal Retired Forest Ecologist	44.74	0.70°W	12.61	7.81	17.47	845.06
*17	Badalona Spain	Cristina Magruga Andreu Universitat Autónoma de Barcelona	41.44	2.18°E	15.41	11.14	19.74	334.13
18	Costa de Santo André Portugal	Adelaide Clemente University of Lisbon	38.10	8.79°W	16.61	12.51	20.76	656.79

* Site from which seed successfully germinated and was used in the study.

Figure 7.2 Location of heathlands in Europe from which seed was sourced for use in the present study.

Climatic Research Unit (Jones *et al*, 2012). The mean annual temperature across the sites ranged between -2.33 to 16.61°C, and annual mean rainfall ranged between 334.13 to 2071.88 mm (Table 7.1).

Collection methods varied between sites but generally seeds were collected when they appeared ripe. Samples were air dried for 24 – 48 h at room temperature before being shipped to the laboratory in Nottingham. All samples were stored at *c.* 5°C for a maximum of 18 months.

7.2.2 Plant culture

In March 2011 *C. vulgaris* seeds were germinated on tap-water agar at 16°C for eight weeks. Seedlings were then transplanted into 160 ml plant pots; the bottom of the pots were lined with 5 x 5 cm 30 µm woven nylon mesh (Clarcor, Lockertex, NY/MO/30/19) and filled with *c.* 150 ml water-washed horticultural silver sand. Plants were grown in the non-mycorrhizal state but with a NH_4 - N based nutrient supply formulated by Stribley & Read (1976) and Hewitt (1966) and optimised for the growth of *Vaccinium macrocarpon* in either the the mycorrhizal and non-mycorrhizal state. Seedlings were watered by addition of excess nutrient solution to the sand every two days (Table 7.2).

Table 7.2 Macro- and micronutrient composition of complete ammonium-type nutrient solution administered to *C. vulgaris* seedlings. Modified after Hewitt (1966) and Stribley & Read (1976).

Element	Salt	Weight (g 100 l^{-1})	Concentration (mM)
Ca, Cl	$CaCl_2$	8.100	0.73
N, S	$(NH_4)_2SO_4$	9.580	0.72
Mg, S	$MgSO_47H_2O$	9.000	0.37
Na, P	$Na_2HPO_412H_2O$	13.170	0.37
K, S	K_2SO_4	6.360	0.37
Fe	Fe citrate $3H_2O$	1.600	0.05
B	H_3BO_3	0.290	0.05
Mn, S	$MnSO_44H_2O$	0.200	0.009
Zn, S	$ZnSO_47H_2O$	0.045	0.002
Cu, S	$CuSO_45H_2O$	0.040	0.002
Mo, Na	$Na_2MoO_42H_2O$	0.025	0.001

Pots were placed in one of three growth rooms each providing a different temperature treatment. Mean annual temperature increase predictions for north-east Europe made by Solomon *et al*. (2007) ranged from 2.3°C to 5.3°C between 1980 and 2100. The mean daily temperature in the United Kingdom between May and August for the period 2001 - 2010 was 14.87°C. Therefore a 16 hr light / 8 hr dark regime was selected with temperatures based on these data (Table 7.2). Mean daily temperatures were increased by 3°C and 6°C to simulate the lower and upper temperature increases predicted in Solomon *et al*. (2007).

Table 7.3 Details of the 16 hr light / 8 hr dark regimes for the three growth rooms in which *C. vulgaris* was assayed.

Temperature Regime	Mean light temperature (°C)	Mean dark temperature (°C)	Mean daily temperature (°C)
T_0	17.00	10.00	14.67
T_{+3}	20.00	13.00	17.67
T_{+6}	23.00	16.00	20.67

Relative humidity was maintained at approximately 80 % by placing a Faran HR-15 centrifugal atomizing humidifier (Faran Industrial Co., Korea) in each room, and a photosynthetic photon fluence rate of *c.* 140 µmol m^{-1} s^{-1} (over the waveband 200-400 nm) was maintained at soil level throughout the light period from a bank of 12 fluorescent tubes. Fifteen pots were grouped in blocks in seed trays which were rotated within the growth room at weekly intervals to control for light and temperature gradients. Each seed tray contained plants from 11 different locations and sites were distributed in a regular and sequential manner throughout the population of 236 pots.

Calluna vulgaris seedlings were harvested after nine weeks growth. Roots were washed free of sand, plants were divided into roots and shoots and then oven dried for 24 h at *c.* 80 °C, and weighed.

7.2.3 Statistical Methods

SigmaPlot 11 (Systat Software Inc. California, USA) was used to perform standard statistical analyses and all data were subjected to normality and homogeneity of variances tests. Relationships between temperature variation and *C. vulgaris* W_W, S_W, R_W and R_W:S_W were investigated using linear regressions, ANOVAs and Pearson's Product Moment correlations. For multiple comparisons in the ANOVAs significance thresholds were corrected using the Dunn-Ŝidák method and *t*-tests used for contrasts.

7.3 Results

Calluna vulgaris seed germinated successfully in 12 of the 21 collections available. Seedlings developed in all temperature treatments, and *n* at harvest varied between 1 and 10 across all sites and treatments. W_W ranged from 0.76 to 44.34 mg, S_W from 0.67 to 39.44 mg, R_W from 0.087 to 7.36 mg, and R_W:S_W from 0.036 to 0.34.

The Dunn-Ŝidák treatment reduced the significance threshold to 0.025. The ANOVAs revealed that elevating temperature from T_0 to T_{+3} did not result in a significant difference in W_W (Figure 7.3 and Figure 7.4a; DF = 24, *P* = 0.028) or S_W (Figure 7.3 and Figure 7.4b; DF = 45, *P* = 0.029). Further temperature increase to T_{+6} also had no significant effect on W_W, S_W or R_W (Figure 7.4a, 7.4b and 7.4c; DF = 24, *P* = 0.076, DF = 24, *P* = 0.048 and DF = 24, *P* = 0.730, respectively). R_W:S_W was significantly lower when the temperature was elevated to T_{+6} (Figure 7.4d; DF = 24, *P* = 0.005). R_W:S_W did not differ significantly between T_0 or T_{+3} (DF = 24, *P* = 0.761).

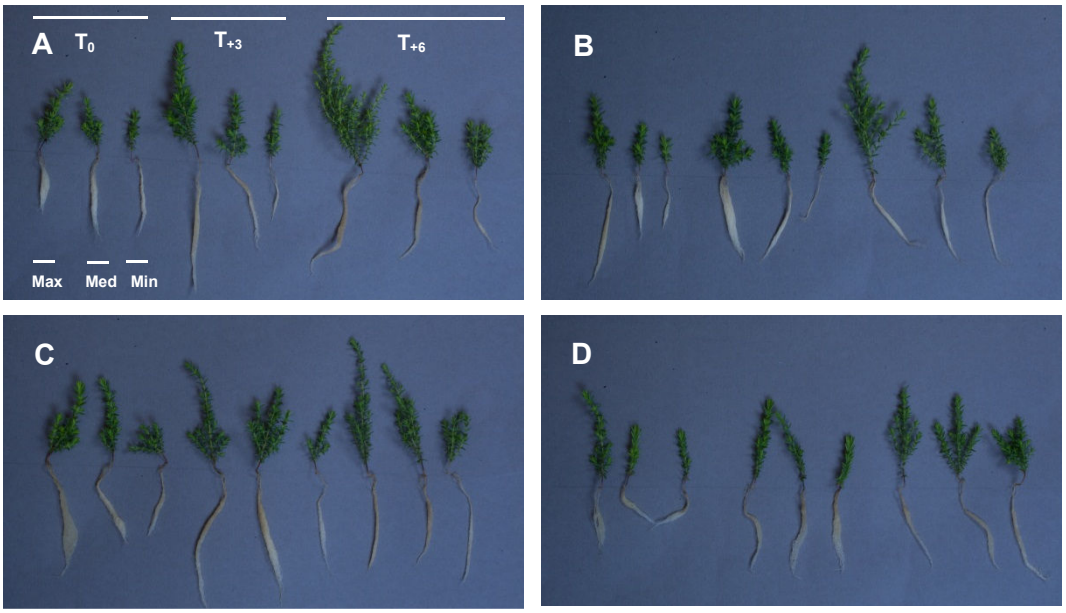


Figure 7.3 Examples of *Calluna vulgaris* individuals judged by eye to be roughly representative of the maximum, median and minimum sizes observed at the three temperature treatments at (A) Kevo Strict NR, Finland (-2.33 °C), (B) Remøya, Norway (5.39 °C), (C) Navazo, Spain (9.08 °C), and (D) Cestas, France (12.61 °C). The temperatures in parenthesis are mean annual temperature at origin for each site.

Relationships between plant growth (W_W, S_W, R_W and R_W:S_W) and TO_{min}, TO_{mean}, TO_{max} and rainfall at origin were tested (Table 7.4). Relationships were strongest when tested against TO_{max} except in R_W at the T_0 and T_{+6} treatments. There was a significant negative relationship between W_W and S_W and TO_{max} in the T_{+3} treatment (Figure 7.5a and 7.5b; r = -0.619, P = 0.04, DF = 11 and r = -0.656, P = 0.02, DF = 11, respectively). R_W decreased with increasing temperature at origin in all cases, but no relationships were statistically significant (Table 7.4). R_W:S_W was positively related to TO_{max} under the T_{+3} (r = 0.605, P = 0.04, DF = 11) and T_{+6} (r = 0.642, P = 0.02, DF = 11) treatments. There was no relationship between temperature and rainfall at origin (r = -0.420, P = 0.175, DF = 104).

Table 7.4 Pearson Moment correlation values (*r*) between *Calluna vulgaris* whole plant mass (mg), shoot dry-mass (mg), root dry-mass (mg) and root:shoot mass ratio at the three temperature treatments, and minimum, mean and maximum temperatures (°C) and mean annual rainfall (mm) across the 11 study sites.

*** - correlation is significant at the *P* ≤ 0.05 level** **** - correlation is significant at the *P* ≤ 0.01 level**

	W_W			S_W			R_W			R_W:S_W		
	T_0	T_{+3}	T_{+6}	T_0	T_{+3}	T_{+6}	T_0	T_{+3}	T_{+6}	T_0	T_{+3}	T_{+6}
TO_{min}	-0.358	-0.512	-0.451	-0.365	-0.541	-0.470	-0.310	-0.275	-.0301	-0.083	0.520	0.406
TO_{mean}	-0.379	-0.573	0.526	-0.387	-0.605*	-0.547	-0.324	-0.306	-0.354	-0.082	0.559	0.479
TO_{max}	-0.389	-0.619*	-0.557	-0.415	-0.656*	-0.582*	-0.252	-0.318	-0.352	0.154	0.605*	0.642*
Rainfall	0.557	0.541	0.815**	0.555	0.516	0.815**	0.546	0.622*	0.783**	0.295	0.053	-0.019

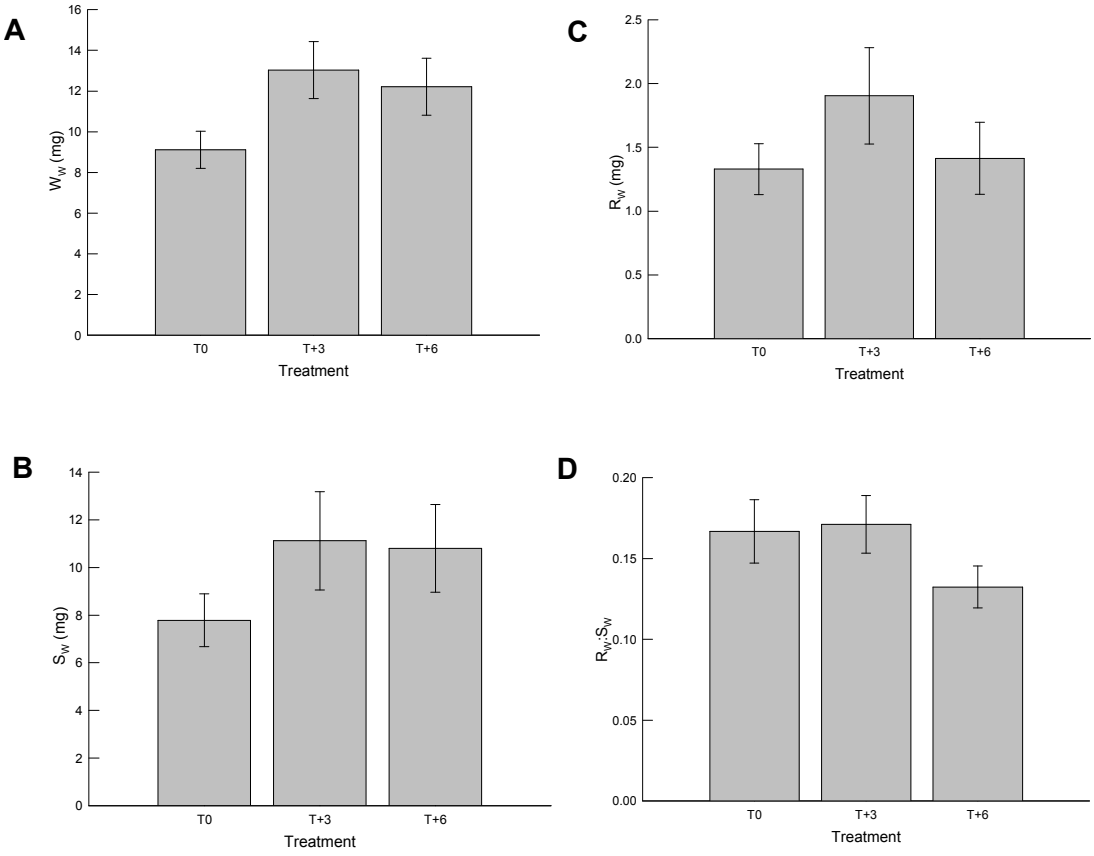


Figure 7.4 The relationship between temperature treatment and (A) *Calluna vulgaris* whole plant dry-mass, (B) shoot dry-mass, (C) root dry-mass, and (D) root:shoot mass ratio across 11 heathland sites. Plotted values are means (*n* = 73 - 81) ± 1 SEM.

There was no relationship between any of the temperature variables tested and the percentage change in W_W, S_W, R_W or R_W:S_W between the T_0 and T_{+3} and T_0 and T_{+6} treatments (Figure 7.6).

W_W and S_W in the T_{+6} treatment (r = 0.815, P < 0.01, DF = 11 and r = 0.815, P < 0.01, DF = 11, respectively), and R_W in the T_{+3} (r = 0.622, P = 0.03, DF = 11) and T_{+6} (r = 0.783, P < 0.01, DF = 11) treatments were positively related to mean annual rainfall at origin (Figure 7.7; Table 7.4).

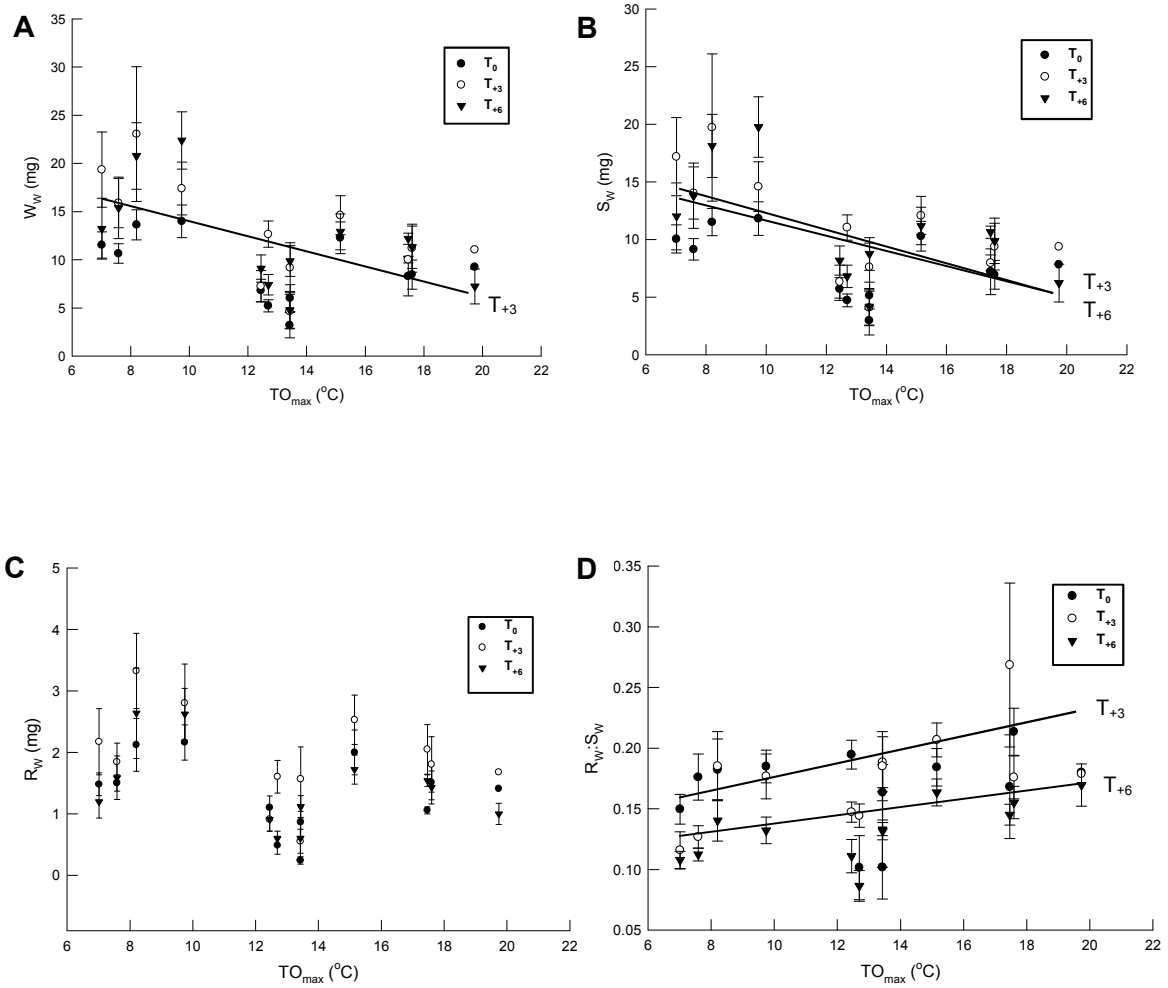


Figure 7.5 Relationships in *C. vulgaris* between maximum annual temperature at origin and (A) whole plant dry-mass, (B) shoot dry-mass, (C) root dry-mass, and (D) root:shoot mass ratio using data for the three temperature treatments. Plotted values are means (*n* = 1 - 10) ± 1 SEM, and lines represent linear regression models.

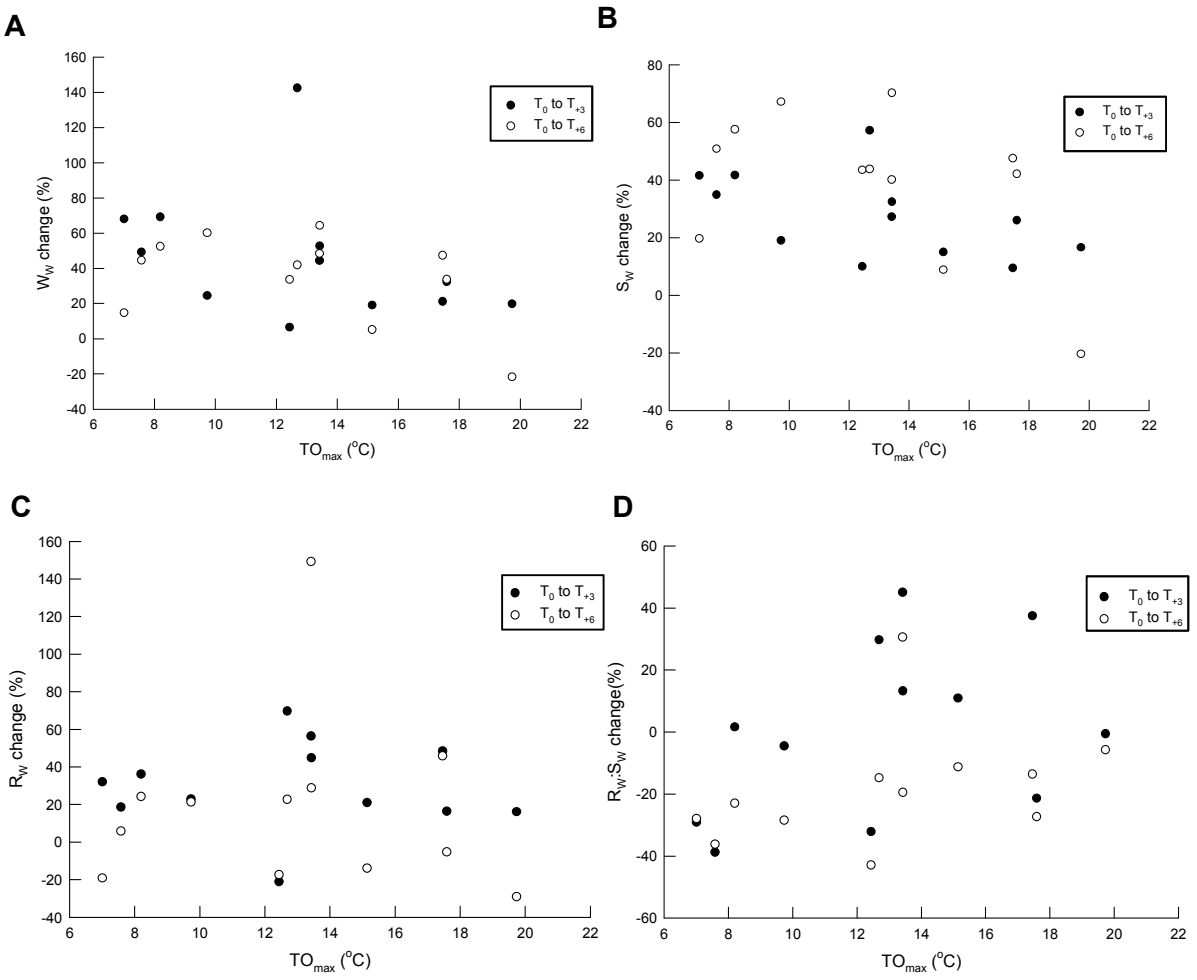


Figure 7.6 Relationships in *C. vulgaris* between maximum annual temperature at origin and (A) whole plant mass, (B) shoot dry-mass, (C) root dry-mass, and (D) root:shoot mass ratio percentage change at the T_0 and T_{+3} and the T_{+0} and T_{+6} treatments.

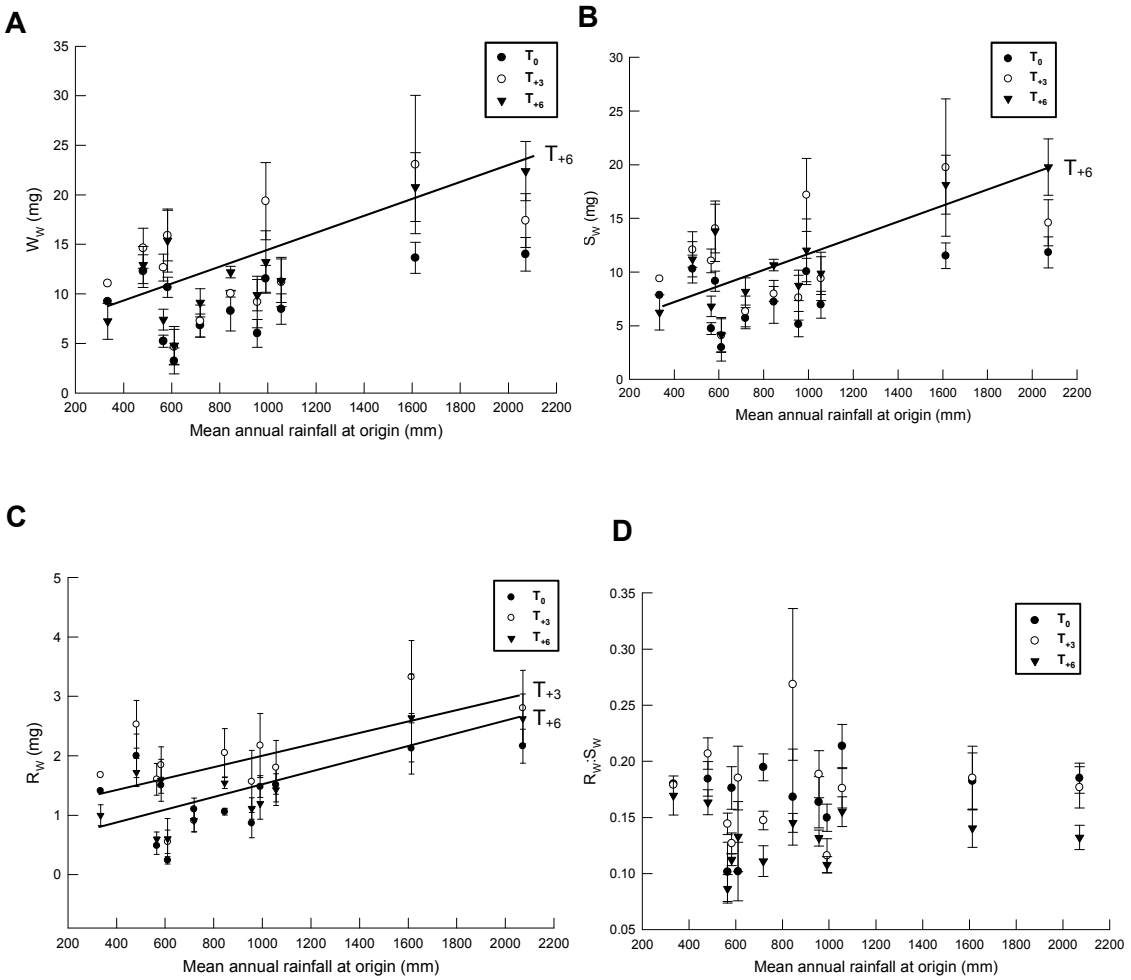


Figure 7.7 Relationships in *C. vulgaris* between mean annual rainfall at origin and (A) whole plant dry-mass, (B) shoot dry-mass, (C) root dry-mass, and (D) root:shoot mass ratio across the three temperature treatments. Plotted values are means (n = 1 - 10) ± 1 SEM, and lines represent linear regression models.

7.4 Discussion

Calluna vulgaris occurs across an extensive latitudinal range in Europe and grows in a wide variety of climates. Global surface temperature is predicted to increase by between 2.3 and 5.3°C by 2099 (IPCC, 2007). This study indicates that under growth room conditions *C. vulgaris* W_W, S_W and R_W respond positively, but not significantly, to warming of 3°C (T_{+3}) above the current annual mean for the UK (T_0), but that an increase of 6°C (T_{+6}) is supra-optimal. R_W:S_W was significantly lower at T_{+6} than at the other temperature treatments. Barber *et al*. (2000) found a strong negative relationship (*r* = -0.520) between increase in temperature and yield in Alaskan white spruce over a 90 year period, and attribute this response to temperature-induced drought stress. Weis & Berry (1988) suggest that yield in plants at extremely high (*c.* > 30 °C) temperatures is limited by photosynthetic functions, including reduced efficiency of primary photochemical reactions in leafs.

This study presents evidence that there is genetic variation in *C. vulgaris* across a latitudinal gradient in Western Europe. W_W decreased significantly with increasing TO_{max} and S_W decreased significantly decreased with increasing TO_{mean} and TO_{max} under the T_{+3} treatment and with increasing TO_{max} under the T_{+6} treatment. R_W decreased with increasing temperature at origin under all treatments, but no relationships were statistically significant. It is accepted that plants from colder environments have higher metabolic activities, such as increased mitochondrial respiration (Körner & Larcher, 1988; Stewart & Bannister, 1974). In the present study, W_W was higher in populations originating from sites with lower TO_{max}. In the case of *C. vulgaris,* an increased growth rate in regions of higher temperatures might be disadvantageous as this would increase susceptibility to drought conditions. Power *et al.* (1998), for example, noted that water loss from *C. vulgaris* shoots

increased under N enrichment, but that increased root mass could compensate for greater water requirement. In the current study, R_W:S_W increased significantly under the T_{+3} and T_{+6} treatments when related to TO_{max}. This suggests that *C.vulgaris* from warmer climates has adapted to increased drought potential by reducing shoot yield, and by increasing R_W:S_W.

Populations originating from regions with lower mean temperatures, conversely, appear to have adapted to produce higher growth rates in order to achieve substantial shoot yield in cooler climates and shorter growing seasons. *Calluna vulgaris* growth is limited at its northern most extent by low temperatures (Loidi *et al.,* 2010), therefore it seems reasonable to suggest that with increased temperatures the climatic envelope of *C. vulgaris* dominated heathland could extend further north under a climate change scenario, providing that the increase in temperature does not exceed 6°C.

There is a known strong link between seed mass and yield in higher plants due to greater initial nutrient availability resulting in larger seedlings (Gross, 1984; Stanton, 1984; Westoby *et al.*, 1992). In the present study it was not possible to measure initial seed or seedling size due to limited seed availability at some sites. The percentage increase in W_W, S_W and R_W between the T_0, T_{+3} and T_{+6} treatments should be independent of seed mass and give an indication of whether plant physiology is driving the relationships observed. There was no relationship between percentage change in W_W, S_W, R_W or R_W:S_W and TO_{max} at any of the treatments investigated. This suggests that seed mass may be affecting yield, rather than physiological processes, but does not negate the possibility of genetic variation in this species.

There was a positive relationship between mean annual rainfall at origin and W_W and S_W under the T_{+6} treatment, and R_W under the T_{+3} and T_{+6} treatments.

There was no relationship between temperature at origin and annual rainfall. Manel *et al*. (2012) found that temperature and precipitation at origin were the primary climatic predictors of genetic variation in 13 alpine plant species sampled across the European Alps. Linhart & Grant (1996) suggest that environmental factors such as soil moisture, temperature and light intensity often vary together and so it can be difficult to determine which of these variables are contributing to genetic variation. For example, even when soil moisture is sufficient, temperature may limit growth, and plants may have adapted to maximise growth capacity in these conditions. In a study of three perennial grass species in a desert with precipitation of 365 mm y^{-1}, Robertson *et al*. (2009) found that under elevated precipitation scenarios growth was determined not only by total precipitation, but also by the intervals between precipitation events. This response was found to vary substantially between species with the growth of *Opuntia phaeacantha* being positively correlated with small frequent precipitation events, and *Bouteloua curtipendula* growth being correlated with larger less frequent precipitation events (Robertson *et al*., 2009). The positive relationships found in the current study indicate the possibility of adaptation in *C. vulgaris* to precipitation levels, although it is unclear if this is a response to total precipitation or the interval between, and magnitude of, precipitation events. As it is predicted that the distribution and magnitude of precipitation events are likely to alter under a climate change scenario, with increases of up to 16% in precipitation expected in northern Europe, and decreases of up to 27% in southern Europe (IPCC, 2007), further research is required to determine the extent to which these factors are driving this apparent genetic variation in *C. vulgaris.*

This study has shown that the growth of *C. vulgaris* varies negatively with TO_{max} across its climatic envelope. However, plants sourced from all locations

responded positively to increased temperature up to 3°C above the current mean annual temperature in Britain, whereas there is evidence to suggest that an increase in 6°C is supra-optimal. This could indicate that there is genetic variation in *C. vulgaris* populations across a latitudinal gradient in Europe, with shoot yield decreasing with increasing temperature at origin. These findings could inform our understanding of the location of the climatic envelope for *C. vulgaris* heathlands under a climate change scenario. In the cooler northern-most parts of its climatic envelope an increase in 3°C might not be detrimental to growth, and *C. vulgaris* could migrate north with a temperature increase of this magnitude. Replicates sourced from the southern-most extent of the range of *C. vulgaris*, however, where growth might be limited by drought, also responded positively to an increase in 3°C, but water was provided in excess, so further study is required to assess the impact of drought at higher temperatures.

8

General Discussion

8.1 Discussion

8.1.1 Soil fertility and vegetation composition

The present study has shown that heathland soil fertility in lowland Britain is related to nitrogen deposition. While such a relationship has been strongly implicated in the results of controlled fertilisation experiments conducted at single locations, this is the first regional survey showing a relationship between N deposition and heather growth rate. Controlled experiments have tended to use large N additions of up to 120 kg N ha^{-1} y^{-1} (Pilkington *et al*., 2005c; Power *et al*., 2004). The N deposition gradient examined here (1.86 - 10.90 kg N ha^{-1} y^{-1}), however, represents an input lower than the minimum N treatment employed by most studies and which frequently failed to produce significant results within this range. The relatively low rate of N enrichment has, however, probably been occurring in many parts of Eastern England for several decades, and this work has provided evidence that this continuous N deposition is enriching heathland soils.

While N enrichment appears to have raised soil fertility, there was no evidence to suggest that this was associated with obvious changes in plant community composition. Several field observations from the British Isles and the Netherlands have strongly suggested that N enrichment promotes growth of graminoids and can result in heathland loss (eg. Aerts & Berendse, 1988; Heil & Diemont, 1983). Sala *et al*. (2000) predicted that elevated N deposition will become the most influential driver of biodiversity loss in temperate forest

zones by 2100. Among the 25 heathlands studied here, graminoid cover ranged from 0 to 76.5%, but not all graminoid rich sites were in high N deposition regions. Coverack, in SW England, and Skipwith Common, in Yorkshire, are two notable examples. There are probably several reasons for this lack of coupling between increased soil fertility and vegetation change. First, because most heathlands are now regarded as having high conservation value, they are being managed to control fertility and successional changes; management can greatly modify the effects of N enrichment. Second, I have shown here that heathland fertility is also related to patch size, possibly due to the ingress of nutrients from the surrounding matrix.

8.1.2 Mitigating impacts of management

Previous research has indicated that heathland management strategies can substantially influence soil nutrient levels and alter vegetation composition (Haerdtle *et al*., 2006; Grant *et al*., 1987; Forgeard & Frenot, 1996). Mitchell *et al*. (2000) and Haerdtle *et al*. (2006), for example, attempted to estimate the number of years of atmospheric N that could be removed by a single application of grazing, sod-cutting or burning. Due to variation in N inputs and site conditions, such as soil moisture, the estimates produced varied by over 150 years of N inputs. The current investigation was unable to quantify the impact of management on soil fertility or vegetation composition. The records kept by landowners proved to be incomplete and unreliable. Burning, for example, is usually applied in small areas in rotation. It was not possible, therefore, to ascertain the burning history at the specific locations from which soil samples were taken, or vegetation composition recorded. Even spatial variation in soil fertility can alter the effectiveness of management, such as burning or mowing (Davies *et al*., 2010). In a heathland at Cannock Forest, Staffordshire, the author of the current investigation observed dramatically

different results from an identical application of burning, with *C. vulgaris* regenerating effectively in one area, and graminoids dominating in another area of close proximity. At this time it is unclear what caused such a dramatic difference in the response of the plant community after burning.

Cursory observations by this author over the course of the current investigation confirmed that management techniques do indeed seem to have a substantial impact on vegetation composition. Graminoid presence, for example, appeared lower where sites had been grazed or sod-cut. The effect of management on soil fertility, as indicated by *C. vulgaris* growth reported in Chapter 3, can be seen when sites subjected to similar nitrogen deposition values, but different management techniques, are compared (Figure 8.1).

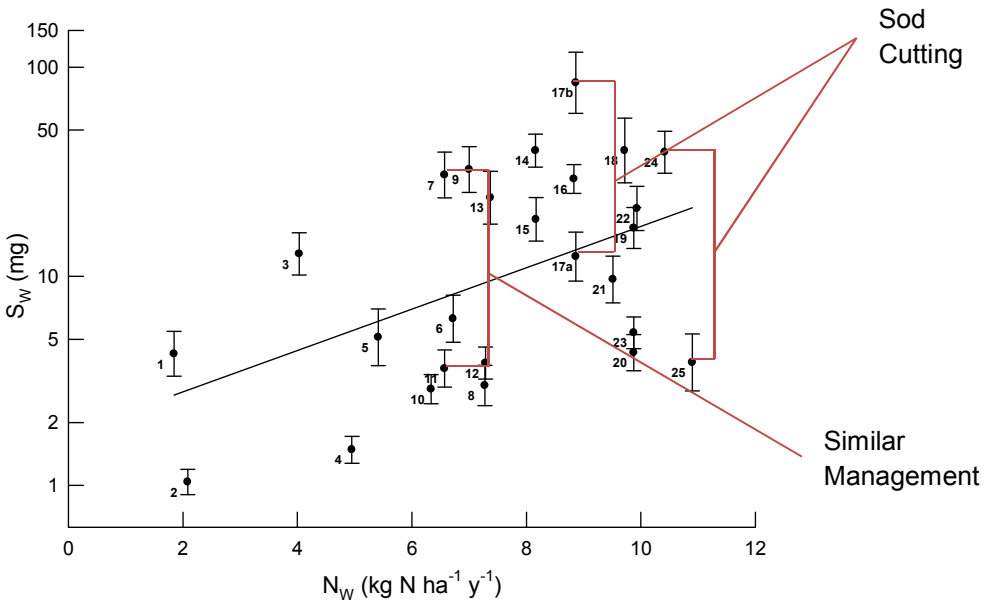


Figure 8.1 Relationship between shoot dry-mass of *C. vulgaris* seedlings and annual mean wet N deposition (N_w) at the 25 study sites. Plotted values are means for each site (n = 10) ± 1 SEM. Red lines indicate comparisons between sites that have been subject to sod cutting and those that have not, and two sites with apparently similar management and N_W values but differing soil fertility.

It can be seen that *C. vulgaris* shoot mass can be substantially reduced by the application of sod-cutting (Figure 8.1). It is, however, difficult to draw conclusions from this as two sites with similar management and nitrogen deposition can also exhibit substantially different soil fertility as indicated by shoot mass values.

The findings of this study can potentially have a number of implications for future heathland management strategies. It was discovered that there is a trend for soil fertility to increase with nitrogen deposition despite the range of wet nitrogen deposition values being restricted relative to the national range. This was, however, not reflected in any aspect of vegetation composition, including species richness or diversity. This is contrary to what is suggested by previous research on grasslands by Stevens *et al.,* 2004, woodlands by Bobbink *et al*. (2008) and Kirby *et al*. (2005), and in heathlands by Maskell *et al*. (2010) and Power *et al*. (1995). It is possible that the nitrogen deposition range used in the current study was not sufficient to identify signals of nitrogen induced vegetation community change. It is more likely that management is effectively mitigating the effects of greater soil fertility. This work suggests that management intervention should continue in order to ensure that elevated soil fertility does not promote the succession of heathland into grassland or woodland.

8.1.3 Heathland patch size

The current investigation found a trend for smaller heathlands to be more fertile, but again, this was not reflected in a relationship with vegetation composition. Piessens *et al*. (2006) reported an increase in soil fertility penetrating 8 m into heathland patches adjacent to cropland and woodland, and this phenomenon may have contributed to the relationship reported in the

present study. Piessens *et al*. (2006), however, also reported a substantial change in vegetation composition at the habitat edge, with reduced presence of *C. vulgaris* and greater presence of graminoids. It is possible that management intervention is mitigating the effect of increased fertility at patch edges in the heathlands studied here by selectively removing unwanted species, such as *P. aquilinum* for example. Piessens *et al*. (2005) suggest that increased patch isolation is a more important driver of heathland deterioration than reduced patch size, since local extinctions could be prevented by dispersal from nearby patches. As the current study did not sample all heathland patches, coupled with the lack of a complete mapped record of the entire heathland resource, it was beyond the scope of this project to determine if patch isolation is a more important determinant of plant species composition.

This work suggests, however, that smaller patches may be more susceptible than larger patches to succession into grassland or woodland due to increased soil fertility, particularly if the site is not managed. Heathland once extended over several million hectares in Western Europe, but estimates suggest that only 350,000 ha remained by 1996 (Diemont *et al*., 1996). Since the realisation by conservationists that heathland has become such a rare habitat there has been a drive to maintain and restore the heathland resource. It is unclear at present whether this has successfully halted the decline in heathland area or reduced fragmentation. In their equilibrium theory of island biogeography MacArthur & Wilson (1967) proposed that the number of species in an island is determined by a balance between immigration and extinction, that extinct species will be replaced by the same or different species through immigration, and that immigration and extinction rates are affected by the island size and its isolation from similar islands. In the context of this work an island can be defined as a heathland patch in a 'sea' of

woodland, grassland or intensive agriculture. Without management intervention this theory might apply to the heathlands studied in the current work, as graminoids and trees may replace ericaceous species more readily in smaller isolated heathland islands. This study, however, suggests that the species-sorting paradigm, in which local abiotic factors affect species extinctions and interactions (Chase & Leibold, 2003; Leibold, 1998; Tilman, 1982), might also apply to heathlands as the ingress of nutrients, including those other than N, from the surrounding matrix might be increasing soil fertility in smaller patches, and consequently could be resulting in reduced heathland patch size as graminoids and tree species competitively exclude ericoids.

The trend for increasing soil fertility with reduced patch size discovered in the present work can inform heathland management. Intervention may need to be more intensive in smaller heathlands where soil fertility is elevated. Habitat corridors may be able to increase immigration in order to counter local extinctions (Beier & Noss, 1998; Debinski & Holt, 2000), but this will not limit elevated soil fertility due to the ingress of nutrients from the surrounding matrix. It is proposed, therefore, that increasing the size of fragments could increase the sustainability of heathland patches, and reduce the need for intensive management intervention.

8.1.4 Nitrogen and phosphorus relationships

While the current work provides evidence of nitrogen enrichment of heathland soils it found no evidence that this led to phosphorus limitation. It is now well documented that experimental additions of nitrogen to a range of habitats, including acid and calcareous grasslands, forests and heathlands, leads to an increase in N:P ratios in vegetation and the up-regulation of

phosphomonoesterase (PME) activity in soil/root systems, bryophytes and lichens (Hogan *et al*., 2010b; Johnson *et al*, 1998 & 1999; Phoenix *et al*., 2003; Phuyal *et al*., 2008; Treseder & Vitousek, 2001). Further, Hogan *et al*. (2010b) showed that N:P ratios and PME activity in the heathland lichen *Cladonia portentosa* varied regionally in Britain and were highly positively correlated with N deposition. These physiological changes have been interpreted as an unfavourable shift in cellular N and P stoichiometry associated with an increase in the efficiency of scavenging P from organic sources via PME. In the present study however, these changes in chemistry and enzyme activity were not seen. In the more fertile heathland soils there was an increase in the uptake of both N and P illustrated by data for both total uptake and tissue concentrations, resulting in a minimal shift in N:P ratio. Further, there was no evidence of increased PME activity in the more N-rich soils. Jones *et al*. (2012) also found no response of soil PME activity to N deposition within the range 13.3 to 30.8 kg N ha^{-1} y^{-1}, but did find a response in litter, which is likely to have higher PME activity values. At the heathland sites used in the current work nutrient enrichment from surrounding vegetation and agricultural activity is likely to be adding P as well as N, and this could partially explain why P reserves appear to be sufficient across the N deposition range studied, and why there was no need for elevated PME activity.

N and P stoichiometry is an important factor to consider when managing heathlands. Roem & Berendse (2000) found that *Molinia caerulea* is able to competitively exclude ericoids to a greater extent when P availability is low. This can result in a greater presence of graminoids which are better adapted to low P availability (Fujita *et al*. 2010; Kirkham, 2001). At present the sources of P, and their extent, are unknown. At heathland sites subject to greater N

deposition values, it is possible that N induced P limitation could contribute to the competitive exclusion of ericoids by graminoids, and thus could lead to heathland loss. The role of PME activity in mitigating the effect of N induced P limitation could become more important in heathland sites with greater N deposition values beyond those studied in the current investigation.

8.1.5 Measures of N deposition

This study used modelled estimates of the wet deposition of NH_4^+ and NO_3^- as the primary measure of N enrichment. Although these are modelled values they are based on interpolations of measured N deposition. Hogan *et al*. (2005b) found that regional variation in total N concentrations and PME activity in *C. portentosa* were highly correlated with both modelled and measured wet deposited NH_4^+ and NO_3^- but that of the two, modelled data were slightly better correlates. Modelled values for total N deposition include highly derived estimates of the dry deposition of NO_x and NH_3 based on modelled interpolated values of atmospheric concentrations. Where in the present work N deposition components other than wet deposited NH_4^+ and NO_3^- were incorporated into regression models for soil fertility, they did slightly improve the predictive power of the models. Throughout this study wet deposited nitrogen data were considered to be more accurately modelled and were used in data analysis.

Ammonia concentration in particular is recognised as a challenging pollutant to model because of the diffuse low level characteristics of the emission sources. Indeed I have shown in the present study that while the lower modelled values of NH_3 pertaining to 5 x 5 km grid squares in which heathlands are located agree closely with measured values at the sites, there

is a clear trend for the higher modelled values to underestimate the field situation (Figure 8.2).

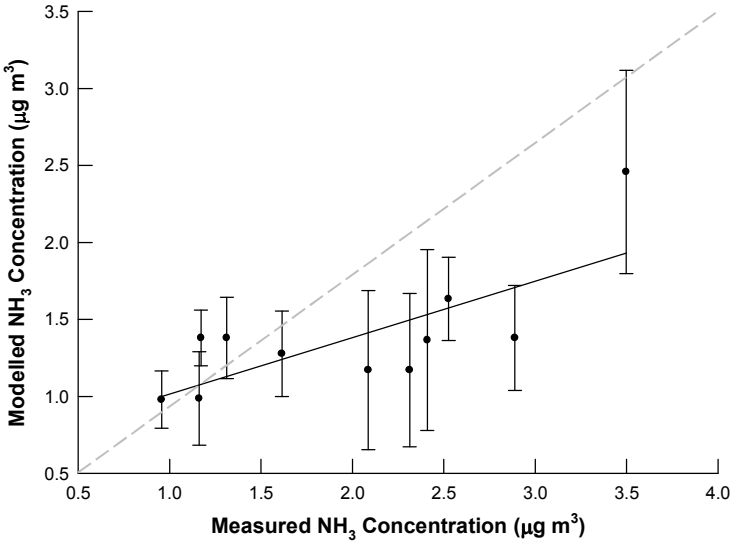


Figure 8.2 Relationship between measured and modelled atmospheric NH_3 concentrations at 11 heathland sites in the East Midlands. Plotted measured data are mean monthly values recorded at 1.5 m above ground level during 2011, and plotted modelled data are mean annual values for the period 2000 - 2008 ± 1 SEM (*n* = 12). The solid line represents the linear regression model and the dashed line represents unity.

These findings are corroborated by those found in other studies. Sutton *et al*. (2001) found that current models may underestimate emissions from livestock such as sheep, for example. Dragosits *et al*. (2002) reported that 5 km resolution models underestimate emissions due to spatial variability in NH_3 sources, and that current models could underestimate critical load exceedances in agricultural areas. Both studies conclude that 5 km resolution models cannot accurately model NH_3 concentrations. Due to the small number of samples available in the current investigation the use of these data for model validation purposes should be cautious. It should, however, prompt further investigations into the accuracy of NH_3 concentration modelling in order to improve said models at a 5 x 5 km grid square scale.

8.1.6 Heathland sustainability under a climate change scenario

This study revealed evidence of significant negative relationships between *C. vulgaris* shoot mass and temperature at origin along a latitudinal gradient in Western Europe. This has possible implications for the future climatic range of *C. vulgaris* under a climate change scenario. Loidi *et al*. (2010) suggest that low temperature limits the occurrence of heathland in the northern most extent of its range, and summer drought limits its occurrence in the south. It therefore seems possible that the area of climatic suitability may extend northward under a scenario of global temperature increase. This current study suggests that adaptations in *C. vulgaris* from colder climates confer a capacity for strong increases in growth in response to warming to a greater extent than populations from warmer climates. *Calluna vulgaris* may therefore respond well under a climate change scenario in the northern-most extent of its range. Temperature increases might also benefit species such as *P. aquilinum*, which is currently considered to be problematic in Britain. *Pteridium aquilinum* exhibits a greater growth response to higher temperatures than *C. vulgaris* (Werkman & Callagham, 1999). Annual precipitation is expected to decrease by between 4 and 27% in southern Europe by 2099 (IPCC, 2007). The current study found a trend for decreasing growth in *C. vulgaris* with lower rainfall at its origin. It is likely, therefore, that decreased rainfall would be detrimental to this key heathland species in the southern-most extent of its range. If summer drought was to become more frequent in southern parts of the climatic envelope under a climate change scenario then this could result in a northward migration of the area of climatic suitability for *C. vulgaris*.

8.1.7 Heathlands in the East Midlands

The investigation intended to place sites located in the East Midlands in a national perspective by comparing heathlands in the East Midlands with 14 sites selected across mainland Britain. This provided a broader range of nitrogen deposition values than was represented in the East Midlands alone. In the current study 11 of the 25 study sites were located in the East Midlands, and therefore they contribute substantially to the trends observed. Wet nitrogen deposition values for the East Midlands heathlands ranged between 6.67 to 9.90 kg N ha^{-1} y^{-1}, which is at the upper end of the range for all sites in the present study. Cursory observations revealed substantial variation in the extent of invasion by graminoids, *P. aquilinum* and trees, such as *Betula pendula.* All sites in the East Midlands were subject to some form of management, but at some sites, such as *Ulverscroft,* which is managed using cattle and selective removal of invasive plants, this seems insufficient to prevent invasion by undesirable plant species. This study has revealed that N deposition alone cannot explain heathland loss due to vegetation composition change and that even within a very small N deposition range heathland vegetation composition can vary substantially. The findings of this investigation suggest that local factors, such as management, may be more important determinants of vegetation composition in the East Midlands heathlands. This highlights the importance of maintaining management regimes to prevent continuing heathland loss.

8.1.8 The need for this research

A number of observations prompted the work reported in this thesis. The link between artificial, often unrealistic, applications of N to heathlands and an increase in soil fertility is well understood, as is the change in vegetation

composition resulting from nutrient enrichment (see Chapter 1). Prior to this research, however, there was very little quantitative evidence of these relationships being reported in heathland systems experiencing natural N inputs, as well as the interaction between the many other factors affecting natural systems, such as management and patch size. The novel approach used in the current study, in which plant growth was used as a bioassay system to assess the impact of pollutants on soil fertility, allowed the study of the impact of sustained N enrichment on natural heathland soils.

8.1.9 Further investigations

This study has yielded a number of questions which require further attention. It is understood that increased soil fertility can result in vegetation composition change and habitat loss in heathlands. The link between N deposition and increased soil fertility has been confirmed by this study, but there was no evidence to suggest that vegetation composition has responded to nutrient enrichment. This is possibly due to the mitigation effect of management practices on the impact of N enrichment on vegetation composition. The method of surveying vegetation in the current investigation, whereby a 2500 m^2 area was sampled, however may have been affected by heathland patch size. Piessens *et al*. (2006) found that soil fertility was greater in an area penetrating 8 m into heathland patches adjacent to cropland and woodland. *Calluna vulgaris* was less dominant and had been replaced by graminoids at the patch edge. In larger heathlands the current study may not have sampled from patch edges and would therefore have not detected the effect of ingress of nutrients from the surrounding matrix which is increasing soil fertility. In smaller patches on the other hand, the entire heathland may have been within 8 m of the surrounding matrix, and consequently affected by nutrient ingress. Therefore it would be interesting to investigate the change of vegetation

composition and soil fertility from the patch edge to the centre of the heathland in order to discover whether nutrient ingress is more influential than atmospheric N deposition at driving vegetation composition and soil fertility changes.

As discussed previously, it is predicted that nitrogen emissions will fall over the next century. Coupled with a substantial drive for heathland conservation and restoration, as well as significant protection from loss due to land-use change, it is possible that heathland habitats are now under a reduced threat of loss or fragmentation. This work has shown that N deposition is increasing soil fertility, and that there is a trend for smaller patches to have higher soil fertility. Revisiting the heathlands studied at a later date would provide evidence of temporal changes under continued management and reduced N deposition, and would allow an assessment of the levels of threat to heathland sustainability.

8.2 Conclusions

This study has revealed significant relationships between N deposition and soil fertility, as indicated by *C. vulgaris* shoot mass, N and P concentrations. This is despite studying a restricted wet N deposition range relative to the full national UK range of 1.86 - 40.40 kg N ha^{-1} y^{-1}. There was no evidence of N induced P limitation, and this could not be explained by up-regulation of soil PME activity. Contrary to expectations, vegetation composition was not related to any variable tested, including modelled N deposition values, heathland patch size or *C. vulgaris* growth or tissue chemistry. Cursory observations revealed that management is clearly affecting vegetation composition and could be effectively reducing soil fertility. This was, however, not possible to quantify and requires further research. Heathland once extended over several

million hectares in Western Europe, but estimates suggest that only 350,000 ha now remain (Diemont *et al*., 1996). This loss is attributed to land-use changes and N-induced succession into woodland or grassland (Rose *et al*., 2000). Matejko *et al*. (2009) found that between 1990 and 2005 total N deposition in Britain decreased from 369 Gg-N to 317 Gg-N, and this reduction is expected to continue. There is a substantial drive for heathland conservation and restoration, and so long as management strategies are implemented effectively, it seems that N-induced heathland loss could slow in the future if N deposition continues to fall. The climatic range of heathland, however, could be modified if temperature increase exceeds 6°C under a climate change scenario. This study has contributed to the knowledge of pollution and temperature events on heathland ecosystems, and has the potential to inform future management strategies.

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Appendix 2


OPAL
Guide to
Heathlands in the East Midlands
Nightjar
Caprimulgus europaeus
Woodlark
Lullula arborea
Tree Pipit
Anthus trivialis
Stonechat
Saxicola rubicola
Linnet
Carduelis cannabina
Great Grey Shrike
Lanius excubitor
Green Woodpecker
Picus viridis
Common Lizard
Zootoca vivipara
Adder
Vipera berus
Grass Snake
Natrix natrix
Slow-worm
Anguis fragilis
Birds
Reptiles

Small Heath Butterfly
Coenonympha pamphilus

Small Copper Butterfly
Lycaena phlaeas

Green Hairstreak Butterfly *Callophrys rubi*

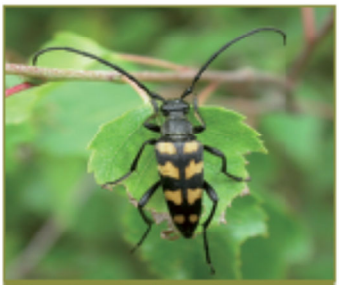
Common Heath Moth
Ematurga atomaria

Emperor Moth
Saturnia pavonia

Brown Silver-line Moth
Petrophora chlorosata

Clouded Buff Moth
Diacrisia sannio

Beautiful Yellow Underwing Moth *Anarta myrtilli*



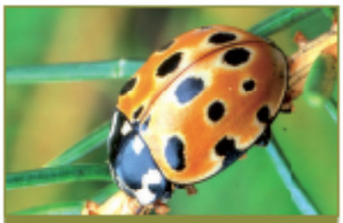
Four-banded Longhorn Beetle *Leptura quadrifasciata*

Minotaur Beetle
Typhaeus typhoeus

Green Tiger Beetle
Cicindela campestris

Garden Chafer
Phyllopertha horticola

● Butterflies and moths ● Beetles



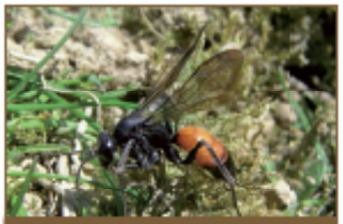
Eyed Ladybird
Anatis ocellata

Ashy Mining Bee
Andrena cineraria

Bracken Sawfly
Aneugmenus padi

Red-banded Sand Wasp
Ammophila sabulosa

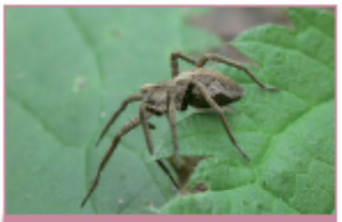
Sand-tailed Digger Wasp
Cerceris arenaria



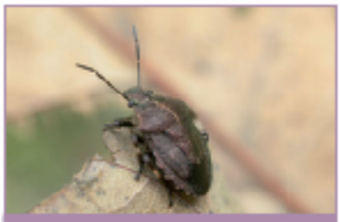
A Spider-hunting Wasp
Priocnemis perturbator

Mottled Grasshopper
Myrmeleotettix maculatus

Four-spot Orb Weaver Spider
Araneus quadratus

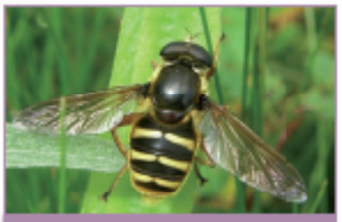


Nursery Web Spider
Pisaura mirabilis



Heather Shieldbug
Rhacognathus punctatus

Birch Shieldbug
Elasmostethus interstinctus



Bog Hoverfly
Sericomyia silentis

Thick-headed Fly
Phyocephala rufipes

Striped Millipede
Ommatoiulus sabulosus

Variegated Centipede
Lithobius variegatus

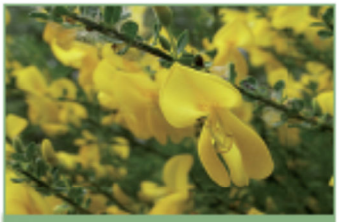
Bees and wasps · Grasshoppers and spiders · Bugs and flies · Myriapods

Heather / Ling
Calluna vulgaris

Cross-leaved Heath
Erica tetralix

Bell Heather
Erica cinerea

Gorse
Ulex europaeus



Broom
Cytisus scoparius

Bilberry
Vaccinium myrtillus

Heath Bedstraw
Galium saxatile

Round-leaved Sundew
Drosera rotundifiora

Tormentil
Potentilla erecta

Rosebay Willowherb
Chamerion angustifolium

Hare's-foot Clover
Trifolium arvense

Wood Sage
Teucrium scorodonia

Wild Thyme
Thymus polytrichus

Sheep's Sorrel
Rumex acetosella

Common Stork's-bill
Erodium cicutarium

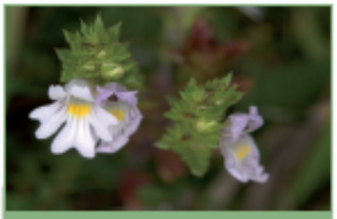
Plants

Heath Dog-violet
Viola canina

Viper's Bugloss
Echium vulgare

Harebell
Campanula rotundifolia

Heath Milkwort
Polygala serpyllifolia



Eyebright
Euphrasia sp.

Field Mouse-ear
Cerastium arvense

Bramble
Rubus fruticosus

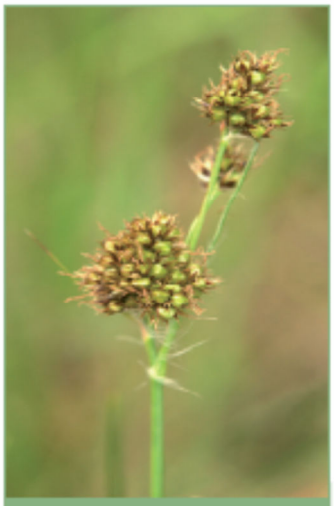
Foxglove
Digitalis purpurea

Bracken
Pteridium aquilinum

Silver birch
Betula pendula

Scots Pine
Pinus sylvestris

Plants



Heath Wood-rush
Luzula multiflora

Red Fescue
Festuca rubra

Carnation Sedge
Carex panicea

Wavy Hair-grass
Deschampsia flexuosa

Neat feather-moss
Pseudoscleropodium purum

King Alfred's Cakes
Daldinia concentrica

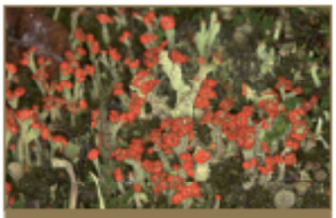
Vermilion Waxcap
Hygrocybe miniata

Orange Birch Bolete
Leccinum versipelle

Moor Club fungus
Clavaria argillacea

Mealy Pixie-cup
Cladonia chlorophaea

Reindeer Moss
Cladonia portentosa



Gritty British Soldiers
Cladonia floerkeana

Plants Lichens and fungi

What is heathland?

Lowland heathland is an open habitat, found less than 300 m above sea level, usually dominated by heathers and different grasses. Larger shrubs, such as gorse and broom, are often present, as well as scattered trees such as birch. Lichens, fungi and mosses can be common on the ground.

Heathlands are typically associated with sandy, acidic, low nutrient soils. Sites vary from large open areas to small fragments within woodland. Heathlands need careful management to prevent succession to woodland, which may involve techniques such as tree removal, grazing, controlled burning and the chemical treatment or rolling of bracken.

Why are heathlands important?

Heathlands are a nationally rare habitat and are home to a large number of rare and unusual species, consequently they are of considerable biological and historical importance. They are particularly important habitats for reptiles and invertebrates and a number of threatened bird species are associated with heathlands, including the European Nightjar.

Threats to the future of heathlands

Heathlands in the East Midlands are typically small and highly fragmented. Threats to heathlands include loss due to urbanisation, afforestation, conversion to agriculture and damage through inappropriate use (e.g. by dirt-bike riders and rubbish tippers). Nitrogen pollution (the deposition of nitrogen-containing compounds emitted into the atmosphere by burning fossil fuels and intensive agricultural practices) is changing heathland vegetative composition by encouraging the growth of less desirable, faster growing species.

Heathlands in the East Midlands

East Midlands heathlands have a unique mix of flora and fauna and are of considerable national importance. The geological diversity in the region results in an unusual juxtaposition of dry and wet heath communities, some of which occur at the extremes of their distribution in the UK[1]. In the 18th Century, vast expanses of heathland existed in the East Midlands, particularly in the Coversands of north Lincolnshire and the medieval Sherwood Forest. However, over the last 200 years the East Midlands has lost over 90% of its heathlands[1].

Nationally, heathland is a priority habitat and many landowners are working actively to improve existing heathlands and create new areas of heathland. There are many volunteer and Friends groups that you could join to help to maintain these fragile habitats. Alternatively why not make a list of the heathland species that you see and send it to your Local Records Centre?

Sites to visit in the East Midlands

Heathlands are at their most attractive between July and September, when many of the plants are in flower. Bear in mind that ground nesting birds are particularly susceptible to disturbance between May and August. When visiting heathlands, remember that they are rare and fragile habitats and many are designated Sites of Special Scientific Interest (SSSIs).

Sites within the East Midlands where heathland is present are listed in the next panel. If you are intending to visit with a group please contact the land owners before you go.

[1]Clifton S.J., and Keymer, R.J. (2009) The lowland heaths of the East Midlands. *Journal of Practical Ecology and Conservation* Special Series, **5**, 48-61.

Name of site	Location	Contact	Facilities
Black Rocks Country Park	SK 295 554	Forestry Commission	P, picnic, toilets, £
Carver's Rock SSSI	SK 330 226	Derbyshire Wildlife Trust	P, V
Eddlestow Lot	SK 323 632	Derbyshire County Council	P, picnic
The Fabrick	SK 356 637	Derbyshire County Council	V
Highoredish	SK 353 596	Derbyshire County Council	P, picnic
Wessington Green	SK 366 572	Wessington Parish Council	P, V
Bagworth Heath Woods	SK 457 068	Leicestershire County Council	P, V, picnic
Beacon Hill SSSI	SK 512 147	Leicestershire County Council	P, V, picnic, toilets, £
Bardon Hill	SK 460 130	Aggregate Industries	V
Kirkby Moor SSSI	TF 225 629	Lincolnshire Wildlife Trust	P, V
Linwood Warren SSSI	TF 133 877	Lincolnshire Wildlife Trust	P*, V
Moor Farm SSSI	TF 226 635	Lincolnshire Wildlife Trust	P, V
Scotton Common SSSI	SK 873 985	Lincolnshire Wildlife Trust	P, V
Harlestone Heath	SP 721 646	Northamptonshire Wildlife Trust	P*, V
Bestwood Country Park	SK 555 476	Nottinghamshire County Council	P, V, picnic, toilets
Budby Heath	SK 614 691	Thoresby Estate	P*
Clumber Park	SK 629 752	National Trust	P, V, picnic, toilets, £
Oak Tree Heath SSSI	SK 568 604	Mansfield District Council	P, V
Rainworth Heath SSSI	SK 593 590	Nottinghamshire Wildlife Trust	P*, V
Sherwood Heath LNR	SK 647 675	Newark and Sherwood District Council	P, V, picnic, toilets
Sherwood Pines Heaths	SK 611 625	Forestry Commission	P, V, picnic, toilets, £
Spalford Warren SSSI	SK 834 681	Nottinghamshire Wildlife Trust	P*, V
Strawberry Hill Heath SSSI	SK 570 603	Nottinghamshire Wildlife Trust	P*, V
Vicar Water Country Park	SK 588 627	Newark and Sherwood District Council	P, V, picnic, toilets

Derbyshire Leicestershire Lincolnshire Northamptonshire Nottinghamshire

P Parking (* roadside or nearby parking only) V Conservation volunteer programme Picnic area Toilets £ Charges apply (e.g. parking)

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Appendix 3 Details of rejected heathland sites.

Site Name	Grid Ref	5 x 5 km square	Modelled Rainfall (mm yr^{-1})	Modelled inorganic N deposition (kg ha^{-1} yr^{-1})†	Reason for Rejection
Hartmount North	NH758782	275E 875N	793	2.0	Replicates site in same 5 x 5 km grid square (Heathmount North)
Heathmount East	NH775788	275E 875N	793	2.0	Replicates site in same 5 x 5 km grid square (Heathmount North)
Heathmount West	NH763786	275E 875N	793	2.0	Soil moisture too high. *Sphagnum* spp. dominant
Moss of Burracks	NH722741	270E 870N	843	2.2	Soil moisture too high. *Sphagnum* spp. dominant
Hill of Nigg	NH827713	280E 870N	779	2.2	No *C. vulgaris* present
Morrich Moor	NH835845	280E 880N	836	2.4	Safety concerns
Lethen Bar	NH953502	295E 850N	838	3.7	Soil moisture too high. *Sphagnum* spp. dominant
Beachy Head	TV565955	555E 095N	690	4.6	No *C. vulgaris* present
Axbridge	ST388557	335E 155N	662	5.0	No *C. vulgaris* present
Cambeak	SX135964	210E 095N	810	5.8	Limited organic soil layer
Trelow Downs	SW920685	190E 065N	813	6.3	Managed by burning recently
Budleigh Salterton	SY037839	300E 080N	766	6.6	*U. europaeus* dominant
* Linwood Warren	TF132876	510E 385N	658	8.0	Limited organic soil layer
Longhorsely Common	NZ157923	415E 590N	713	8.4	Soil moisture too high. *Sphagnum* spp. dominant
* Bradgate Park	SK315112	450E 310N	761	9.1	
* Coxmoor Golf Club	SK525575	450E 355N	750	9.3	Extremely limited *C. vulgaris* cover
* King's and Baker's	SK925297	490E 325N	679	9.5	Limited organic soil layer
* Strawberry Hill	SK582604	455E 360N	755	9.9	No *C. vulgaris* present
Berkhampsted Heath	SU995136	495E 210N	761	10.8	No *C. vulgaris* present

† N - NO_3^- + N – NH_4^+

* East Midlands sites